Smooth brome invasion influences nitrogen cycling and soil bacterial community structure in a fescue grassland

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Abstract

Exotic plant invasions represent a significant threat to the integrity of native grasslands. Across the Northern Great Plains, grasslands invaded by smooth brome (*Bromus inermis* Leyss) support lower plant diversity, potentially resulting in important consequences for ecosystem function. Previous research on smooth brome has primarily focused on aboveground changes in plant communities, but there is growing evidence that the soil ecosystem can be significantly altered with invasion. The two objectives of this thesis were to examine whether smooth brome invasion alters soil nitrogen cycling, and to determine if changes in plant community diversity or productivity influence soil bacterial communities. Relationships between smooth brome and the soil ecosystem were assessed using data collected from a *Festuca hallii* Vasey (Piper) (plains rough fescue) grassland located near Macrorie, SK. Gross rates of nitrogen cycling and community productivity from smooth brome invaded and native grassland sites were compared to determine the potential influence of smooth brome invasion on the soil nitrogen cycle. The relationship between increasing smooth brome abundance and soil bacterial structure and composition was also studied. Gross mineralization rates and total soil nitrogen were significantly higher in smooth brome-invaded areas relative to native grassland. Bacterial and archaeal *amoA*, used as indicators of ammonia-oxidizer population sizes, were altered by smooth brome cover. Higher gross mineralization rates were likely due to stimulated microbial activity caused by increased litter and root production in areas invaded by smooth brome. Smooth brome decreased plant species richness through increased litter production, but had

the opposite effect on bacterial communities. Bacterial communities had higher species richness and evenness in soils invaded by smooth brome, and smooth brome invasion was also associated with bacteria important for soil nitrogen cycling. As bacteria dominate microbial biomass and are important for decomposition processes, a more even bacterial community may have supported increased mineralization rates in smooth brome-invaded soils. Specifically, a more even bacterial community may have increased mineralization rates through greater resource utilization and niche partitioning. The responses observed in these studies suggest that belowground changes with smooth brome invasion have the potential to have important consequences for ecosystem processes.

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List of abbreviations

AOA- Ammonia-oxidizing archaea

AOB- Ammonia-oxidizing bacteria

NMS- Non-metric multidimensional scaling

SEM- Structural equation model

1 Introduction

Grasslands are a highly diverse and important ecosystem worldwide, but their continued sustainability is jeopardized by threats such as invasive plants (Mack et al., 2000; PCAP, 2009; White et al., 2000). Invasion of an exotic species causes significant changes to many aspects of plant community structure including biomass and diversity (Ehrenfeld, 2010; Liao et al., 2008). These alterations may in turn cause significant changes to plant-soil relationships and belowground ecosystem functioning. For example, invasive species can alter nutrient cycling (reviewed by Ehrenfeld, 2003) and the composition and structure of the soil microbial community (e.g. Hawkes et al., 2005; Klironomos, 2002; Kourtev et al., 2002). In the Northern Great Plains, invasion of smooth brome (*Bromus inermis* Leyss) reduces native plant species richness and significantly changes aboveground plant community structure (Fink and Wilson, 2011; Otfinowski et al., 2007; Romo et al., 1990). Most research on smooth brome thus far has primarily examined aboveground changes, but there is growing evidence that, like other invasive species, smooth brome alters the belowground ecosystem (Fink and Wilson, 2011; Jordan et al., 2008). These studies highlight the potential importance of belowground processes in smooth brome invasion, but the full role and consequences of altered belowground functioning has not been elucidated. Research in this area is essential to fully understand the legacy of smooth brome in grassland ecosystems.

In this thesis, the influence of smooth brome on the aboveground plant community, and the direct and indirect effects of smooth brome on the soil ecosystem in a fescue grassland were examined. The objectives were to assess if smooth brome invasion influences either the soil nitrogen cycle or the structure of the soil bacterial community, and if so, what the potential mechanisms behind these alterations might be.

This introductory chapter provides a review of relevant literature on grassland ecosystems, an overview of invasive species, a brief description of smooth brome and ends with the study objectives. Following the introduction is the first data chapter where the relationship between smooth brome and soil nitrogen cycling is examined. This study shows that patches of smooth brome have altered nitrogen cycling patterns compared to native grassland, potentially as a result of increased above and belowground organic matter production. The second data chapter examines the influence of smooth brome on soil bacterial community richness, evenness and composition. The results of this study show that bacterial species richness and evenness are higher in smooth brome invaded areas, and that some groups of bacteria, including bacteria important for N cycling, are associated with a changing plant community composition. In the final chapter, the important findings from this research are summarized along with a discussion on future research directions.

1.1 Grassland Communities and Plant-Soil Interactions

Grassland ecosystems are one of the largest and most biologically diverse

ecosystems worldwide (Gibson, 2009). Approximately one-fifth of North America is dominated by grasslands, and is an important reservoir for biodiversity (PCAP, 2009). However, in the last 150 years the extent of natural and semi-natural grasslands has been reduced to ~20% of its former extent, representing the largest loss in land cover of all ecosystems in North America (Hammermeister et al., 2001). In Saskatchewan, only 21% of the Prairie ecozone remains as native grassland, primarily due to conversion to agriculture (Hammermeister et al., 2001). Resource extraction, urban development and invasion of exotic species are also implicated in the loss of native prairie (Hammermeister et al., 2001). Due to this significant loss in land cover, there is a disproportionately high number of endangered and at risk wildlife species inhabiting the remaining grasslands (Gauthier et al., 2003).

Grassland plant community productivity, diversity, and composition are controlled by complex interactions among biotic and abiotic factors. Over large spatial scales, climate and geology are the prevailing factors determining grassland community type, with disturbances such as fire, grazing and drought important at smaller spatial and temporal scales (Gibson, 2009; Gross and Romo, 2010). Within plant communities, above and belowground competition and facilitation may be instrumental in controlling plant community structure (Gibson, 2009). Belowground interactions may be especially important, as the soil is an important source of nutrients and water (Cahill and Lamb, 2007). The importance of these resources is highlighted by the fact that up to 80% of plant biomass is in belowground plant structures (Jackson et al., 1996; Pucheta et al., 2004).

As well as plant roots, the soil matrix harbors an important group of organisms that are highly diverse and extremely important in key ecosystem functions. Soil communities are composed of a vast array of bacteria, archaea, fungi, micro- and macro fauna (Bardgett, 2002). Despite the importance and extremely high biodiversity of these organisms, relatively little is known about the ecology or function of individual species (Coleman and Whitman, 2005). Bacteria and fungi are the primary decomposers in the soil ecosystem (McGuire and Treseder, 2010), but it is unclear how important biodiversity within these groups is to overall ecosystem functioning (Bardgett, 2002; Bell et al., 2005). Some evidence suggests that there are key species whose removal has a disproportionate impact on ecosystem functioning (Bardgett, 2002), while other studies suggest that species richness and evenness are also important determinants of ecosystem function (Bell et al., 2005; Wittebolle et al., 2009).

The soil microbial biomass is typically dominated by bacteria, a highly diverse and species rich group (Fierer and Lennon, 2011). Primary reasons for this high diversity include life history traits allowing for relatively quick evolution, diverse metabolic adaptations, and the use of dormancy to avoid competition (Fierer and Lennon, 2011). In soils, most bacterial species are rare (Elshahed et al., 2008), but there are typical phyla, including the *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*, that are dominant in the soil ecosystem (Bergmann et al., 2011; Fierer et al., 2007). Little is known about most bacterial groups in the soil, but evidence suggests that certain phyla show differing responses to soil properties such as resource availability and pH

(Eilers et al., 2010; Fierer et al., 2007; Jones et al., 2009). Other bacterial groups, such as *Nitrospira* and *Rhizobium* are well known for their functional roles in the nitrogen cycle, although their relationships to soil and plant variables is not well established.

Interactions between plants and the soil microbial community can have important direct or indirect consequences for both communities (Bever et al., 2010). For example, pathogens in the soil can have a negative impact on plant growth, while mycorrhizal symbioses can positively influence plant community growth and regulate plant community diversity (van der Heijden and Horton, 2009; Wardle et al., 2004). Plant communities are indirectly influenced by the soil microbial community, as the release of mineralized nutrients through microbe-driven processes are essential to plant productivity (van der Heijden et al., 2008). Plant community structure, such as species richness, evenness, and composition can also influence the soil microbial community structure through the release of compounds above- and belowground (Bartelt-Ryser et al., 2005; Kielak et al., 2008; Lamb et al., 2011; Sanon et al., 2009; Wardle, 2002; Zak et al., 2003), but see (Cruz-Martinez et al., 2009; Kielak et al., 2008; Porazinska et al., 2003). In particular, changes to root exudate profiles and litter quality and quantity are implicated as potential links between the plant and soil microbial communities (Berg and Smalla, 2009; Haichar et al., 2008; Wardle et al., 2004; Wardle et al., 1999).

Interactions between the plant and soil microbial communities also have important consequences for ecosystem processes such as nutrient cycling. In temperate grasslands, nitrogen, along with water, limits plant productivity (Gibson,

2009; Lamb, 2008; Vitousek and Howarth, 1991). The soil microbe and plant communities exert control over decomposition and mineralization of organic nitrogen (Strickland et al., 2009; Wardle, 2002). Plant communities provide the organic material, such as litter or roots, for decomposition (Couteaux et al., 1995; Craine et al., 2002; Facelli and Pickett, 1991; Wardle, 2002). The abundance and quality of this organic material, as determined by the plant community, influences the composition and activity of the microbial community. The microbial community in turn influences the rates of nitrogen mineralization, as well as the subsequent processes of nitrification and denitrification (Wardle, 2002).

Grassland community structure is controlled by a complex interaction of biotic and abiotic conditions with varying importance at different spatial scales. These conditions and processes have been discussed in the context of a natural ecosystem, but grasslands are facing other, primarily human-induced changes, that also have the potential to alter grassland community structure and function. Invasive species are recognized worldwide for their detrimental impacts on ecosystems, and the next chapter will discuss the causes and consequences of exotic species invasions.

1.2 Invasive Species

1.2.1 Invasive Species

Invasive species are organisms whose establishment and persistence in a new ecosystem results in significant negative consequences, such as reduced biodiversity (Mack et al., 2000). In terms of species loss, these organisms are recognized as a significant threat to biodiversity worldwide, second only to habitat

destruction (Williamson, 1999). Invasive species are also associated with significant economic losses, as a consequence of lost ecosystem services and restoration costs (Pimentel et al., 2005). In Saskatchewan, over 50 invasive plants have been recorded (Saskatchewan Conservation Data Centre, 2012), several of which represent a significant threat to grassland ecosystems (PCAP, 2009). In some cases (e.g. crested wheatgrass, smooth brome), species introduced deliberately to Saskatchewan for forage production have become invasive.

Establishment and invasion of an ecosystem by an exotic species often represents a "perfect storm" scenario, in which a species introduction coincides with an ecosystem that is susceptible to its invasion. Despite the growing number of successful invasions, this "perfect storm" situation is relatively rare (Mack et al., 2000). Most organisms transported to a new location perish upon arrival, or are unable to reproduce (Mack et al., 2000). However, with the increase in human traffic and other human-driven activities such as habitat fragmentation or development of roads, the probability of introducing an invasive species has increased tremendously (Mack et al., 2000). In other cases deliberately introduced species have become invasive (Pimentel et al., 2005; Romo et al., 1990).

1.2.2 Ecosystem susceptibility to invasion

The presence of an invasive species does not necessarily dictate its eventual dominance in the ecosystem, as the susceptibility of the ecosystem to invasion also plays a role. A link is often drawn between establishment of an invasive species and reduced biodiversity, but a causal link between the two cannot always be established (Bauer, 2012; MacDougall and Turkington, 2005). Considerable debate

exists around what makes an ecosystem susceptible to invasion (Lonsdale, 1999). Changes in the natural disturbance regime, for example, through overgrazing or changes in fire frequency (Gibson, 2009), have been associated with weakened resistance to invasion, but what specific factors reduce community resistance are not well understood. One common hypothesis is that communities with higher diversity may be more resistant to invasion than species poor communities (Case, 1990). There is support for this hypothesis (e.g. Tilman, 1997), however, study results are inconsistent and often depend on the scale of the study (Stohlgren et al., 1999). Another theory suggests that disturbances or fluctuations that alter resource availability influence community susceptibility to invasion, rather than any one characteristic of a community (Davis et al., 2000). For example, overgrazing may reduce plant vigor, and result in the resident plant community being unable to utilize all available resources (Davis et al., 2000). These unused resources may then become available to assist in the establishment of an invasive species (Davis et al., 2000). This theory suggests that all ecosystems, regardless of species richness or other factors, may be susceptible to invasion (Davis et al., 2000).

These theories suggest that invasive species may be "passengers" following ecosystem change, establishing in situations where native species are limited in some way (MacDougall and Turkington, 2005). However, invasive species can have direct impacts independent of environmental conditions (e.g. Flory and Clay, 2010), suggesting that some invasive species may be "drivers" of change (MacDougall and Turkington, 2005). At least two studies have explicitly tested and found the ability of invasive species to drive change (White et al., 2013; Wilson and Pinno, 2013). A

variety of mechanisms may allow an invasive species to drive ecosystem change, including plant traits, interactions with the soil microbial community, or changes to ecosystem level functions such as nutrient cycling. These mechanisms will be discussed in the next section. As the objectives of this thesis were to examine the role of belowground processes in invasion, this section will focus more specifically on this aspect of invasion ecology.

1.2.3 Invasion Mechanisms

Much research has examined whether certain plant traits can be used as indicators of invasiveness (e.g. Laungani and Knops, 2009; van Kleunen et al., 2010). In a recent meta-analysis, invasive species were shown to have traits conferring increased survivability and competitiveness compared to native, non-invasive species (van Kleunen et al., 2010). Examples of such traits include greater growth and photosynthetic rates, seed production, germination and survival, biomass production above and belowground, and higher leaf surface area (van Kleunen et al., 2010). These trait differences were consistent across climatic zones, suggesting that similar traits may be important, regardless of host ecosystem (van Kleunen et al., 2010). In ecosystems where nitrogen is limited, traits that confer greater nitrogen use efficiency may be important (Laungani and Knops, 2009). In particular, specific nitrogen use traits may allow invasive species to maintain the high biomass with which they are typically associated (Laungani and Knops, 2009). For example, traits that reduce a plant's N requirement (e.g. higher synthesis of carbon per unit N), or increase the total plant available N (e.g. through increased N residence time or stimulation of soil nitrogen turnover rates) may be competitively advantageous.

A second major invasion mechanism is the enemy release hypothesis (Keane and Crawley, 2002). This theory states that invasive species have escaped from their natural enemies and are less affected by enemies in their host range compared to native species (Keane and Crawley, 2002). This results in a release in competitive pressure to the advantage of the invasive species (Keane and Crawley, 2002). For example, Klironomos (2002) found invasive species exhibited positive growth effects when grown in soils cultured by their own species, compared to rare, native plants that exhibited a negative impact on growth. When *Centauria maculosa*, an invasive plant, was grown in soil from its native range, soil organisms had a negative impact on its growth, but when grown in soil taken from its invaded range, the soil had a positive effect on growth (Callaway et al., 2004).

Invasive species may also influence the soil microbial community through the production of "novel weapons" (Callaway and Ridenour, 2004). This theory suggests that invasive species may release exudates into the soil, which in its native range may have had little consequence for ecosystem function, but in its new range has significant consequences (Callaway and Ridenour, 2004). These novel chemicals can influence either the soil microbial community, with indirect effects on the plant community, or directly influence the plant community through allelopathy (Callaway and Ridenour, 2004). Multiple studies have examined the inhibitory effects of isolated phytochemicals on plants and soil microbes in a lab setting (e.g. Dorning and Cipollini, 2006; Kim and Lee, 2011), but in situ evidence of this theory is lacking. One study by Callaway et al. (2008) found that *Alliaria petiolata* reduced mycorrhizal growth in its invaded range but not in its home range. These effects

were attributed to phytochemicals produced by *Alliaria* but it is unknown how strong these effects would be under natural settings (Callaway et al., 2008).

Through changes in dominant plant traits, and direct and indirect influences on the soil microbial community, invasive species may alter soil nutrient or carbon cycling (Ehrenfeld, 2003). The degree to which an invasive species can influence nutrient cycling or pool sizes may be dependent on how different the invasive plant traits are compared to the native plants (Ehrenfeld, 2003). For example, invasive species with N-fixing capabilities may be less N-limited than native species in low N environments (Vitousek and Walker, 1989). Invasive species often have higher growth rates and productivity, which can influence the size of the plant C pool (Ehrenfeld, 2010). A meta-analysis by Liao et al. (2008) showed that most invasions increase carbon and nitrogen pools above and belowground (but see Wilson and Christian, 1999). In temperate grassland ecosystems, nitrogen is a limiting nutrient (Lamb, 2008; Vitousek and Howarth, 1991), therefore changes in soil nitrogen cycling may have significant consequences for the native plant community.

Invasive species may influence soil nitrogen cycling rates by altering shoot community composition. Shoot composition is a strong determinant of the quality and quantity of litter input to the soil (Facelli and Pickett, 1991; Cornelissen, 1996), which has consequences for litter decomposition. Litter produced by invasive species has been found to differ in quality or quantity from that typical of the local native species (Ehrenfeld, 2003). In particular, litter quality is often higher than native plant litter, resulting in higher mineralization rates (e.g. Vinton and Goergen, 2006). Belowground, changes to the plant root community structure can alter the

composition or quantity of root exudates (Bais et al., 2006). These compounds can influence the abundance and structure of the soil microbial community in invaded and native communities (Hawkes et al., 2005; Inderjit and van der Putten, 2010; Kourtev et al., 2002; Reynolds et al., 2003; Wolfe and Klironomos, 2005) and indirectly influence mineralization rates (Zak et al., 2003).

Interactions with the soil microbial community or with soil nitrogen itself may cause changes to soil nitrification rates. Ammonia-oxidizing bacteria (AOB) and archaea (AOA) are important organisms in the soil, as they catalyze the conversion of ammonium to nitrite, the rate-limiting step in the nitrification process (Kowalchuk and Stephen, 2001). Changes in the plant community structure, such as changes in species richness or evenness, may directly influence the abundance of AOA and AOB via root biomass production, or release of root exudates (Lamb et al., 2011). Alternatively, AOB and AOA population sizes may be altered indirectly by changes to mineralization rates or availability of ammonium for substrate (Okano et al., 2004). In turn, nitrification rates are influenced by changes in AOB and AOA populations, affecting plant available nitrate (Di et al., 2010; Di et al., 2009; Hawkes et al., 2005).

This section reviewed some of the mechanisms and potential consequences of exotic species invasions on a wide variety of species. Smooth brome is a widespread exotic grass invading grassland ecosystems across the prairies (Otfinowski et al., 2007), and is the focus of this thesis. The next section will provide a description and review of smooth brome literature.

1.3 Smooth brome (*Bromus inermis* Leyss)

Smooth brome (*Bromus inermis* Leyss), a perennial, C_3 grass, was introduced to Western Canada from its native range in central Europe around 1888 (Otfinowski et al., 2007). This species was originally introduced to North America to improve hay production, and is widely used for hay production in Saskatchewan (Government of Saskatchewan, 2012). Despite its positive contribution to forage production, smooth brome has established and flourished in a variety of ecosystems, to the detriment of native flora and fauna (Fink and Wilson, 2011; Romo et al., 1990). Smooth brome is now widespread across Canada, invading a range of habitat types from forests to grasslands, and often occurring in disturbed areas such as roadside ditches (Otfinowski et al., 2007). Features such as an extensive rhizome system and prolific seed production aid the establishment of smooth brome into native ecosystems (Grilz et al., 1994; Otfinowski and Kenkel, 2008). Invasion of smooth brome creates dense, monoculture patches ranging in size from 5-15 m^2, but can reach patch sizes of up to 900 m^2, and up to 20 000 m^2 in extreme cases (Otfinowski et al. 2007). In areas of smooth brome invasion, plant diversity can be reduced up to 70% (Otfinowski et al., 2007). Attempts at eradication or management of smooth brome in grasslands have been met with limited success (Bahm et al., 2011; Wilson and Gerry, 1995). In Tallgrass Prairie, burning was effective at controlling smooth brome, but may not be effective in other ecosystem types (Grilz and Romo, 1994).

As shown by the above review, invasive species often have significant impacts on the belowground ecosystem, and can influence important ecosystem

processes such as nutrient cycling. Few studies have directly examined the influence of smooth brome on ecosystem processes, and the observed effects are conflicting. In Saskatchewan, Fink and Wilson (2011) found no change in plant available nitrogen under smooth brome stands. Similarly, Nosshi et al. (2007) found that soil N mineralization rates did not differ between smooth brome and two other native grasses. However, two studies suggest that smooth brome may be better able to capitalize on unused nitrogen relative to native species. In Tallgrass Prairie, smooth brome had higher tiller density and biomass production in experimentally N-enriched soils (Vinton and Goergen, 2006). In a Fescue Prairie, smooth brome invasion was influenced by community disturbance and resulting changes in soil nitrogen availability (Otfinowski and Kenkel, 2010).

Only one study could be found that examined the impact of smooth brome on the soil microbial community, although an earlier report suggested that smooth brome may produce allelopathic compounds (Rice, 1967). In a pot study, Jordan et al. (2008) found that smooth brome, when grown in soil cultured by itself, exhibited a positive growth rate relative to sterilized soil. These effects were attributed to modification of the soil microbial community, and modifications by smooth brome also increased growth of *Euphorbia esula* (leafy spurge) seedlings (Jordan et al., 2008). These studies suggest that smooth brome may be able to cause changes in ecosystem functioning, and a recent study (Wilson and Pinno, 2013) found that smooth brome can drive ecosystem changes in low disturbance or high N environments. In summary, the responses observed in these studies suggest the

possibility of significant consequences to soil ecosystem structure and function in smooth brome invaded areas.

1.4 Objectives

Invasive species such as smooth brome can cause significant changes to its host ecosystem. The objective of this thesis was to examine the impact of smooth brome invasion on the soil ecosystem in a remnant Fescue Prairie. In the first data chapter, the influence of smooth brome on soil nitrogen cycling rates was examined. In particular, the following questions were examined: 1) Do soil nitrogen cycling rates differ between smooth brome-invaded areas and native grasslands, 2) How do changes in plant community productivity, and litter quality relate to altered nitrogen cycling rates, and 3) How do ammonia-oxidizing bacteria and archaea respond to smooth brome invasion? The second data chapter sought to address the relationship between smooth brome and the soil bacterial community, specifically, 1) Is soil bacterial richness and evenness influenced by smooth brome abundance, 2) Does smooth brome influence bacterial richness and evenness through changes in root biomass and soil properties, and (3) Are the abundance of bacterial taxonomic groups altered by the strong changes in plant community composition associated with smooth brome?

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Chapter Preamble

This chapter assesses whether soil nitrogen cycling rates and ammonia-oxidizing bacteria and archaea population sizes differ between native Fescue Grassland and smooth brome-invaded areas. Areas invaded by smooth brome had higher gross mineralization rates and total soil nitrogen compared to native fescue grassland. Gross nitrification rates and ambient nitrate and ammonium did not differ between invaded and native soils. Smooth brome had a weak positive influence on ammonia-oxidizing bacteria and archaea population sizes. Higher mineralization rates were hypothesized to be due to the increased plant biomass production in smooth brome invaded soils. However, because nitrification rates were unaffected, the additional mineralized nitrogen may have been immobilized in the microbial biomass. As nitrogen is a limiting nutrient in temperate grasslands, higher mineralization rates and altered ammonia-oxidizer population sizes highlight an important consequence of smooth brome invasion as well as a potential mechanism of invasion.

This chapter relates to the overall thesis because it studies an important aspect of the relationship between invasive plants and soil ecosystem functioning. In particular, the effect of smooth brome on nitrogen cycling processes, including its effects on ammonia-oxidizing bacteria and archaea, an important soil microbial functional group is examined.

2 Smooth brome alters soil nitrogen cycling processes in a Fescue Grassland

2.1 Abstract

Invasive plant species are a major threat to ecosystems. A variety of invasion mechanisms have been proposed, including the ability of an invasive species to alter nutrient cycling. To investigate the role of altered nutrient cycling in plant species invasions, we studied gross nitrogen cycling rates in a smooth brome (*Bromus inermis* Leyss) - invaded grassland near Macrorie, SK., Canada. The goal of this study was to examine whether nitrogen cycling rates differ between smooth brome invaded and native Fescue Grassland soil, and to examine potential mechanisms for these changes. In particular, potential mechanisms examined included changes to plant community productivity and ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) population sizes. Nitrogen mineralization rates were higher in smooth brome-invaded soils compared with native soils. These higher mineralization rates may be due to the greater quantity of plant biomass being produced both above and belowground in invaded areas. Litter C:N ratio, a measure of litter quality, did not differ between invaded and native areas, suggesting the microbial community may be stimulated by the greater amount of organic material incorporated in the soil via the litter. No change in nitrification rates between invaded and native soils, and only a weak effect of smooth brome on AOA and AOB population sizes were observed. Thus, it appears that smooth brome is stimulating N mineralization rates through the production of increased plant biomass but that this increase is not linked to increases in soil nitrification, possibly

due to high N immobilization rates. Alterations to nitrogen mineralization may be an important mechanism by which smooth brome is able to dominate in its host environment.

2.2 Introduction

Invasive plants are a significant, global threat to many ecosystems as their introduction often displaces native plant species and reduces habitat for native animals (Mack et al., 2000; Vilà et al., 2011). Aboveground changes in plant community structure are most apparent following invasion, reducing both the species richness and evenness of the plant community, while often increasing productivity (Ehrenfeld, 2010; Liao et al., 2008). These changes aboveground are also reflected belowground. For example, invasive species have been implicated in changing the soil microbial community, root distribution in the soil, and other soil properties (Bradford et al., 2012; D'Antonio and Mahall, 1991; Duda et al., 2003; Kourtev et al., 2002; Sanon et al., 2009). These changes, both above and belowground, have significant impacts on ecosystem processes such as nitrogen cycling (Ehrenfeld, 2003; Laungani and Knops, 2009; Liao et al., 2008).

The diversity and composition of a plant community can influence nitrogen cycling rates (Craine et al., 2002; Knops et al., 2002; Scherer-Lorenzen, 2008; Wardle, 2002; Wedin and Tilman, 1990), through changes in plant-based inputs to the soil. Aboveground, shoot community composition is a strong determinant of the quality and quantity of litter input to the soil (Cornelissen, 1996; Facelli and Pickett, 1991), and affects decomposition rates (Couteaux et al., 1995; Knops et al., 2002; Scherer-Lorenzen, 2008; Wardle, 2002). Litter produced by invasive species can

differ in quality or quantity from local native species, altering mineralization rates (Ehrenfeld, 2003). As well, changes in traits such as nitrogen use efficiency or residence time may also influence the rate of nitrogen flow through the plant community (Laungani and Knops, 2009). Belowground, changes to the plant root community caused by the invasive species can alter the composition or quantity of root exudates (Bais et al., 2006; Callaway and Aschehoug, 2000). These compounds influence the abundance and structure of the soil microbial community in invaded and native communities (Bais et al., 2006; Hawkes et al., 2005; Inderjit and van der Putten, 2010; Kourtev et al., 2002; Reynolds et al., 2003; Wolfe and Klironomos, 2005) and indirectly influence mineralization rates (Zak et al., 2003).

An invasive species may influence soil nitrogen cycling through direct interactions with the soil microbial community or through its effects on soil nitrogen itself. For example, the abundance of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) may be directly influenced by species richness or evenness of the plant community via root biomass production, or release of root exudates (Lamb et al., 2011). Alternatively, AOB and AOA population sizes may be altered indirectly through changes to mineralization rates or availability of ammonium for substrate (Okano et al., 2004). In turn, nitrification rates are influenced by changes in AOB and AOA populations, affecting plant available nitrate (Di et al., 2010; Di et al., 2009; Hawkes et al., 2005).

Smooth brome is a widespread, invasive grass throughout Western Canada, where its introduction has resulted in significant losses of biodiversity, up to 70% in some cases (Otfinowski et al., 2007). Smooth brome has higher tiller density and

biomass in experimentally N enriched soils (Holub et al., 2012; Vinton and Goergen, 2006), suggesting the potential for altered N cycling rates in smooth brome-invaded areas. In this study, the first objective was to determine if smooth brome-invaded and native grassland soil differed in nitrogen cycling rates. The second objective was to examine plant community productivity, litter characteristics, and AOA and AOB population sizes as potential factors influencing these altered nitrogen cycling rates (Fink and Wilson, 2011; Otfinowski et al., 2007).

2.3 Methods

2.3.1 Field site

The field site is a 14.6 ha remnant Fescue Prairie undergoing invasion by smooth brome, approximately 120 km south of Saskatoon, SK., Canada (51°12' N 107°17' W), near the border of the Moist Mixed and Mixed Prairie Ecoregions and within the Orthic Dark Brown Chernozemic soil order (Agriculture and Agri-Food Canada, 2010). A total of 65 plant species were found at the site. Dominant native grasses included *Festuca hallii*, and several species of *Hesperostipa, Elymus and Pascopyrum.* A variety of forbs were also abundant. The landscape consisted of rolling hills, with upland species such as *Koeleria macrantha* and *Bouteloua gracilis* occurring on hilltops, and more shrubby species (e.g. *Symphoriocarpus occidentalis*) occurring in lower areas. Smooth brome is invading the site from disturbed edges (primarily roads), and many invasion patches are present in the interior of the site. No management practices are present on the site and have not been for at least 27 years (Jim Romo, personal communication). Average yearly temperature is 3.5 ˚C and the average yearly precipitation for this area is 376.9 mm (Rock Point Weather

Station, ~7 km from field site, Environment Canada, 2012). The sampling year was an average summer, with 204.9 mm of rain falling through the May-August period, and an average temperature of 16.0 °C (Environment Canada, 2011).

2.3.2 Ammonia-oxidizer and plant biomass study

This study was completed during July 2011 at sampling points generated using the random sample point generator in ArcMap (Esri, Redlands, CA, U.S.A.). A stratified random sampling pattern was used to include samples from a range of smooth brome cover classes. A total of 60 locations with 15 samples in each of four categories of aboveground smooth brome abundance were sampled (0%, >0-50%, 51-85% and >85%). At each location, plant community cover was assessed in a 50 x 50 cm quadrat. Plant material was removed and separated into grass, forb, shrub, and litter biomass. Brome biomass was collected separately from other grass. Biomass samples were dried for 2 days at 60˚C and weighed. To determine litter C:N ratio, a subsample of dried litter was ground and analyzed on a Leco AutoAnalyzer (Leco Corp., St. Joseph, MI., U.S.A) for total carbon and nitrogen.

Immediately after biomass collection, soil samples were collected from the A and B horizons. Soil cores were taken near the centre of the quadrat. Two soil cores (5 x 5 cm) per sample point were composited from both the A and B horizons. These samples were frozen at -20 ˚C. From each soil core, all roots were carefully picked out of the soil and frozen separately. As the roots were used for a separate study on root distributions, samples were not dried, and fresh weights are presented. Total C and N for soil samples were determined using a Leco Carbonator (Leco Corp., St. Joseph, MI, U.S.A.).

The abundance of the *amoA* gene, which encodes a region of the ammonia monoxygenase enzyme responsible for the first, rate-limiting step in nitrification, was used as a proxy to measure AOA and AOB population sizes. DNA was extracted from 0.5 g of 2 mm-sieved soil using the Ultraclean Soil DNA extraction kit (MoBio, Carlsbad, CA., U.S.A.), and stored at -20 ˚C until use. DNA concentration was determined using a UV-Vis spectrophotometer (Nanodrop 2000, ThermoScientific, Wilmington, Del. U.S.A.). Q-PCR was used to determine archaeal *amoA* and bacterial *amoA* gene copy numbers, with QuantiTect SYBR Green Master mix (Qiagen) and an ABI 7500 real-time PCR machine. For archaeal *amoA*, the primer set arch-amoAF/AR (Park et al., 2006) and amoA-1F/2R for the bacterial *amoA* assay (Rotthauwe et al., 1997) was used. Total reaction volume was 20 µl and consisted of: 10 µl SYBR Green Master mix, 0.3 µM each primer, 0.2 mg ml^{-1} BSA, 4.8 µl ultrapure water, and 2 µl template DNA. Reaction conditions for archeal *amoA* were 97°C for 15 min, 45 cycles of 94 °C for 20 sec, 54 °C for 40 sec, 72 °C for 40 sec, followed by a data acquisition step at 80 °C for 45 sec. Bacterial *amoA* conditions were 97 °C for 15 min, followed by 40 cycles of 94 °C for 15 sec, 58 °C for 40 sec, 72°C for 30 sec, 78 °C for 45 sec (data acquisition step). Both qPCR runs were followed by a dissociation curve to verify the amplification of a single, pure product.

Standard curves were created using purified PCR products from community DNA and were included in every run. We have validated the use of community DNA standards by comparison to pure *Nitrosomonas europea* genomic DNA standards (Banerjee and Siciliano, 2012). Dilutions of PCR products ranging in magnitude from 10^2-10^8 copies were used for the standard curves; curves were linear over the entire

spectrum. Amplification efficiencies were greater than 90% and r^2 values were 0.99 for both reactions. The potential effect of inhibitory products in the samples was evaluated by spiking standards with sample DNA. No inhibitory effects were identified with the dilution used in these assays. As the copy numbers of bacterial-*amoA* were low (less than 100 copies), we reran samples that had been cleaned a second time to determine if there were inhibitory substances. No difference in C_t values between cleaned and original DNA samples was found. Additional optimization steps did not increase the number of bacterial *amoA* copy numbers, so the numbers presented likely represent the low abundance of ammonia-oxidizing bacteria in this system. Bacterial *amoA* was not detected in seven samples, despite repeated runs. These non-detect samples were converted to the lowest theoretical value possible for qPCR (3 copies) (Bustin et al., 2009) to allow for inclusion in the analysis. Archaeal *amoA* samples were run in duplicate, while only approximately one third of samples were run in duplicate for the bacterial *amoA* assay (all non-detects and low copy numbers were run in duplicate). Gene copies are reported as the average log copy number ng DNA^{-1} g $dry\ soil^{-1}$. For presentation on the figure, different symbols are used for samples that fell below either the standard curve or detection limits.

2.3.3 Nitrogen cycling study

Many studies of invasive species have examined net mineralization or nitrification rates. These measures provide a good estimate of changes in plant available N, but do not examine whether the processes themselves are being changed. Therefore, ^{15}N stable isotope chemistry was used to determine gross

mineralization and nitrification rates, in a field study completed August 8-9, 2011, shortly after (within 2 weeks) of the completion of the biomass and soil study. Typically, green biomass and productivity peak in Fescue Grasslands during July (Redmann et al., 1993), and so the N cycling study was completed after peak biomass to potentially capture the influence of that year's plant inputs on soil N cycling. No dramatic changes in temperature or moisture conditions were observed between late July and early August with plant community biomass remaining active until late August in 2011. Average soil moisture at the site was 12.9 ± 3.6%. To avoid locations that had been disturbed by biomass and soil collection, ArcMap was used to generate different random sampling points, and assigned the samples to one of two treatments: an "invaded" treatment, where the aboveground smooth brome cover was >85%, or to the "native" treatment, where there was no smooth brome present. If the generated sample point was not suitable, the point was moved to the nearest suitable location. Data was collected from 15 invaded and 15 native points, for a total sample size of 30.

At each sampling location, a total of 5 soil cores (5 x 15 cm) were taken using a soil sampler with removable plastic liners (AMS, Inc., American Falls, ID, U.S.A.) (Bedard-Haughn et al., 2006). Litter biomass was removed before sampling, so cores consisted primarily of mineral soil. One soil core was used for the determination of gravimetric water content and ambient nitrate and ammonium levels. For each incubation (mineralization or nitrification), two soil cores were injected seven times with 2 ml of 30 µg/ml N (98% ^{15}N enrichment). Cores used for the mineralization incubation were injected with $(^{15}NH_4)_2SO_4$ and nitrification

incubation cores were injected with $K^{15}NO_3$. Injections were performed using an 18 gauge side-port needle, and were evenly spaced over the soil core to ensure homogeneous distribution of ^{15}N. One core of the pair was capped and placed in the ground for 24 hours before extraction. The other core was extracted within 15 minutes of injection. For extraction, soil cores were homogenized, and a subsample (~20 g) was extracted with 2 M KCl and filtered. Extracts were frozen at -20˚C until analysis.

Atom percentages of ^{15}N were analyzed following Davidson et al. (1991). For mineralization, subsamples of KCl extract were made alkaline with the addition of MgO, converting ammonium to ammonia vapor (Davidson et al., 1991). This vapor was captured by a Teflon sealed acid disc. For mineralization samples, this acidified disk was removed after seven days of shaking and analyzed. For nitrification samples, this first disk was discarded. A second disk was added, along with Devarda's alloy, to convert the remaining nitrate to ammonium. These samples were shaken for an additional seven days and analyzed. All samples were analyzed using a Costech ECS4010 elemental analyzer coupled to a Delta V mass spectrometer with Conflo IV interface in the Department of Soil Science, University of Saskatchewan. To determine nitrate and ammonium, a Technicon Autoanalyzer was used (Technicon Industrial Systems, 1978).

Mineralization and nitrification rates were calculated as outlined in Davidson et al. (1991). For some samples, these calculations resulted in negative values (two mineralization and three nitrification samples). Multiple scenarios could have produced these negative values, including variability across paired samples,

violation of a methodological assumption, or rates so low as to be negligible in the time frame of this experiment. For analysis, any values outside the range of three standard deviations from the mean were treated as erroneous and not included while values within three standard deviations of the mean were considered negligible (zero). This range of standard deviations was calculated without the negative values. For mineralization, one sample was removed from the analysis, and one sample was treated as zero. For nitrification, all three negative values were treated as zero.

2.3.4 Statistical analyses

General linear models were used to test for differences in mineralization and nitrification rates, ambient nitrate and ammonium levels, and mean turnover times for nitrate and ammonia (calculated as gross mineralization or nitrification divided by ambient ammonium or nitrate levels). Models were fit using the glm function in the R package (R Core Development Team, 2012), for each of the above response variables, with "Treatment" as the explanatory variable. Ambient ammonium and nitrate levels were log transformed prior to analysis. The effect of smooth brome on litter biomass, shoot biomass and litter C:N ratio was assessed using a general linear modeling approach (glm function, R Core Development Team, 2012). For total shoot and litter biomass analyses, the only explanatory variable included was percent aboveground smooth brome cover. For the litter C:N ratio analysis, percent legume cover and species richness were also included as explanatory variables. The amount of N being added to the soil via the litter was scaled using total litter biomass and the litter N values. The significance of this relationship was assessed using a general

linear model, with brome cover as the explanatory variable. Changes in root biomass and ammonia-oxidizer population sizes were tested with linear mixed models (Pinheiro et al., 2012), with "Plot" as a random term. Both horizon and aboveground smooth brome cover were included as explanatory variables. Root biomass was log transformed prior to analysis to improve normality. To assess linearity between the *amoA* data and smooth brome cover, a quadratic term was included as an explanatory variable. For both the AOA and AOB models, the quadratic terms were not significant.

2.4 Results

2.4.1 ^{15}N Study

Gross mineralization rates were 37% higher in smooth brome-invaded soils compared to native grassland soils (Figure 2.1). Correspondingly, total soil nitrogen was significantly higher in invaded soils compared to the native soils (Figure 2.1) and was correlated with gross mineralization ($r = 0.65$, $p < 0.001$). There were no differences in gross nitrification rates, ammonium, nitrate levels or soil gravimetric water content (Figure 2.1). Mean turnover rates between treatments did not differ for ammonia ($F_{1, 28} = 1.84$, $p = 0.186$) or nitrate ($F_{1, 28} = 2.55$, $p = 0.121$).

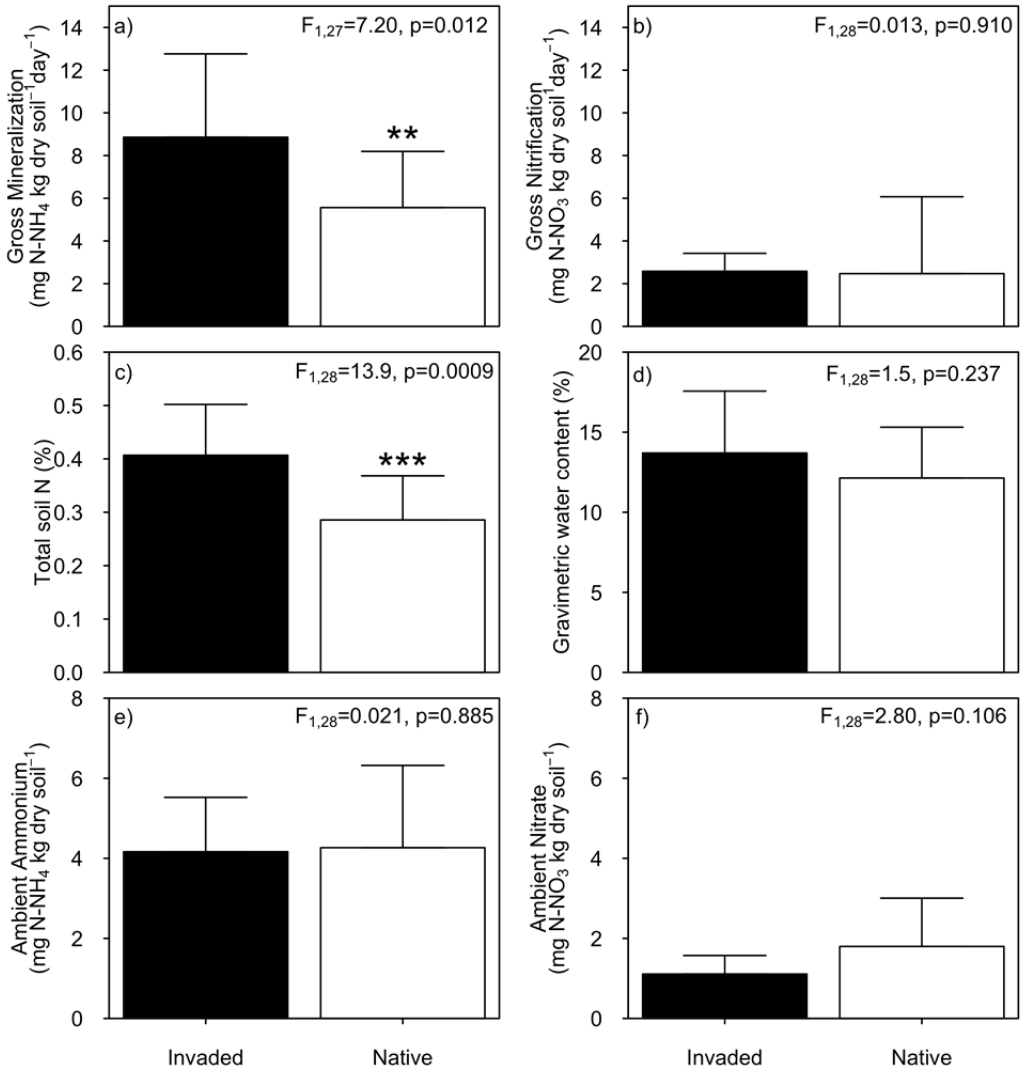

Figure 2.1 Differences in a) gross mineralization rates, b) gross nitrification rates, c) total soil N, d) gravimetric water content, e) ambient ammonium, and f) ambient nitrate between smooth brome invaded and native grassland soils. Bars represent the estimate of the mean of invaded (n=15, except for gross mineralization, n=14) and native (n=15). Invaded soils had >85 % smooth brome cover and native soils had no smooth brome cover. Error bars represent standard deviation around the mean.

2.4.2 Plant Biomass

Smooth brome-invaded areas had greater productivity above and belowground. Total shoot and litter biomass increased with smooth brome cover (Figure 2.2). Litter C:N ratio increased only marginally with smooth brome (Figure 2.2), and there was no effect of species richness ($F_{1, 56}$ =1.14, p=0.28) or legume cover ($F_{1, 57}$=0.15 p=0.69). These two variables were removed from the final model. When the amount of N added to the soil (via the litter) was scaled based on the amount of litter produced, the total litter N increased significantly with smooth brome cover (Figure 2.2). Root biomass was greater in the A horizon than the B ($F_{1,58}$=140.2, p=0.001), and increased with smooth brome cover in both horizons ($F_{1,58}$=10.96, p=0.002) (Figure 2.3). The horizon by brome interaction term was not significant for this model ($F_{1, 58}$=1.35,p=0.25).

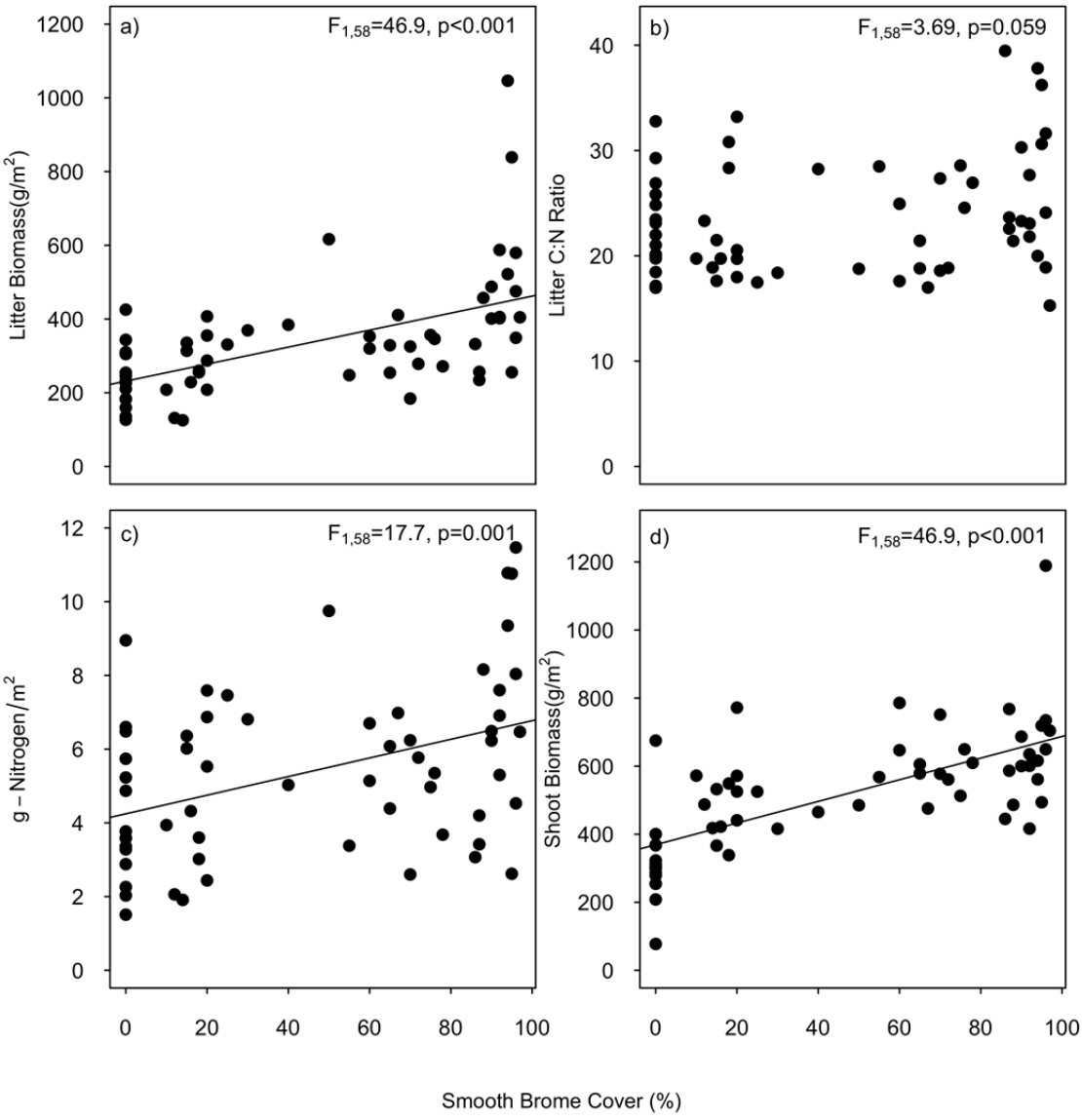

Figure 2.2 Relationship between smooth brome and a) litter biomass (r^2=0.300), b) litter C:N ratio, c) grams of N added to soil via litter (calculated as the percent N of the litter biomass) (r^2=0.166), d) shoot biomass (r^2=0.447).

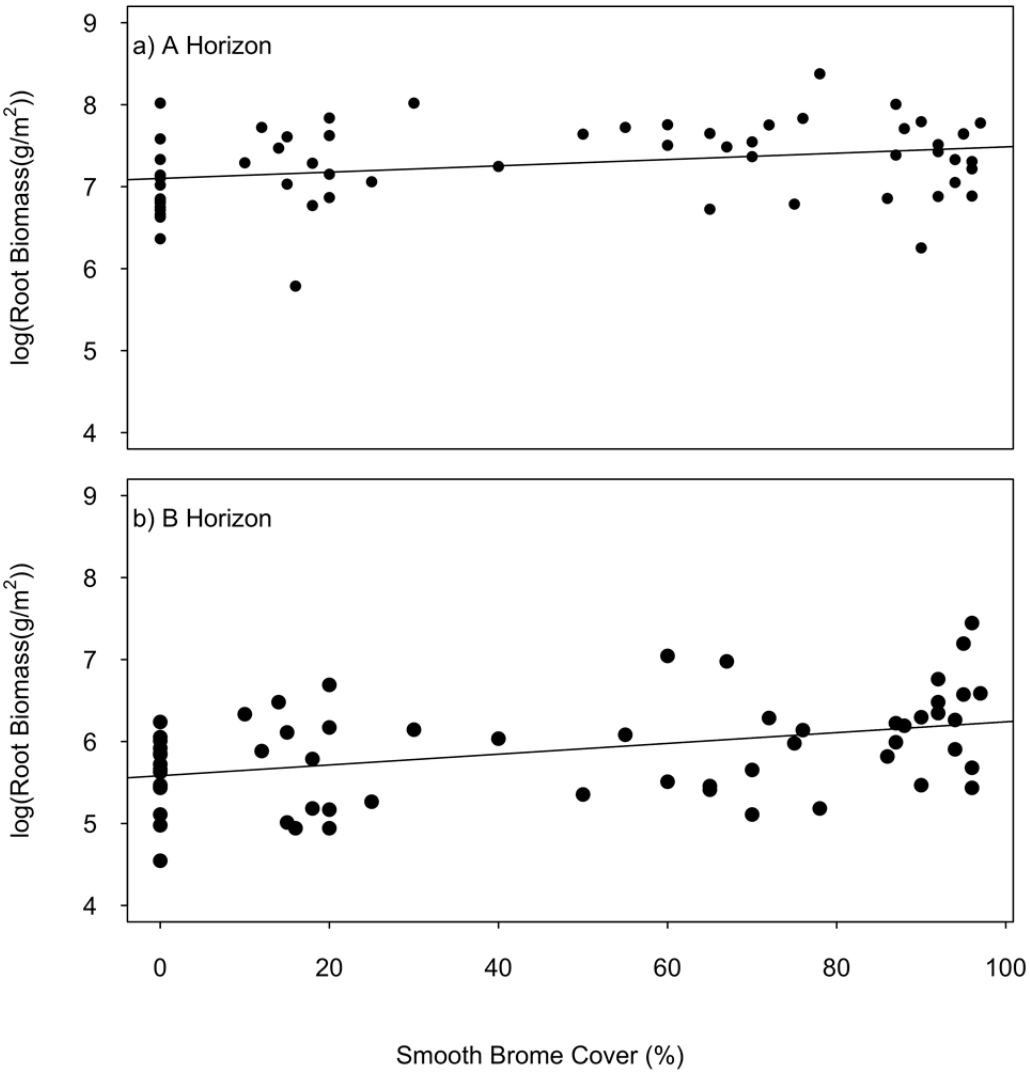

Figure 2.3 Smooth brome significantly increased root biomass in both the a) A Horizon (*log root biomass*=0.005(0.001)x +7.0(0.09) (r^2=0.082) and b) B horizon (*log root biomass*=0.005(0.001)x +5.64(0.09)) (r^2=0.161) .

2.4.3 Ammonia-oxidizing bacterial populations

There was no effect of smooth brome on archaeal *amoA* copy numbers ($F_{1, 58}$ =1.54, p=0.218). A nearly significant ($F_{1, 58}$ =3.95, p=0.052) interaction term between soil horizon and smooth brome cover indicated archaeal *amoA* increased with smooth brome cover in the B horizon (Figure 2.4). In contrast, smooth brome had no effect on archaeal *amoA* in the A horizon. Archaeal *amoA* was also less abundant in the A horizon (3.89±0.50) compared to the B horizon (4.23±0.44) ($F_{1, 58}$ =21.6, p<0.001). For bacterial *amoA*, there was a significant main effect of smooth brome cover ($F_{1, 58}$ =5.57, p=0.022) but the smooth brome by horizon interaction term was not significant ($F_{1, 58}$ =2.22, p=0.142). Bacterial *amoA* increased with smooth brome cover in the A and B horizons (Figure 2.4). Copy numbers of bacterial *amoA* were higher ($F_{1, 58}$ =92.69, p<0.001) in the A (1.86±0.80) compared to the B horizon (0.73±0.77).

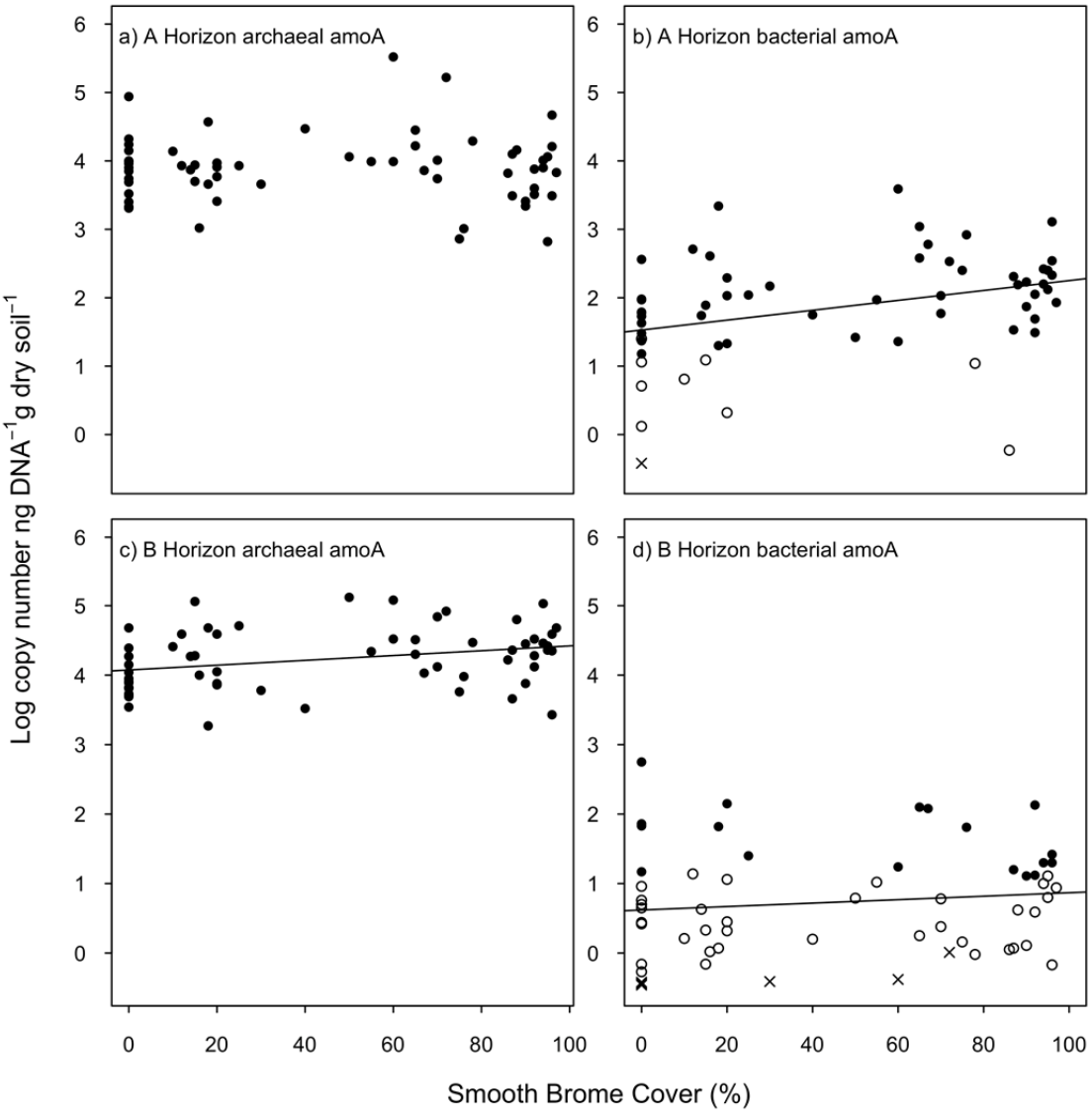

Figure 2.4 Relationship between smooth brome and AOA/AOB *amoA* copy number. Top two panels (a) and (b) represent A horizon data, bottom two panels (c) and (d) are B horizon data. Left panels (a) and (c) are archaeal *amoA* copy numbers and right panels (b) and (d) are bacterial *amoA* copy numbers. For archaeal *amoA*, the regression line is not significant in the A horizon, but is significant in the B horizon (*archaeal amoA*=0.004(0.002)x +4.07(0.11), r^2=0.091). Regression lines in the A horizon (*bacterial amoA*=0.005(0.002)x +1.64(0.14), r^2=0.120) and the B horizon

(*bacterial amoA*=0.005(0.002)x +0.52(0.12), r^2=0.015) are both significant. Closed circles represent values within the standard curve, open circles represent samples below the standard curve, and crosses represent samples that were not detected.

2.5 Discussion

Smooth brome-invaded areas had altered nitrogen cycling processes compared to native fescue grassland. In particular, soils under smooth brome had higher mineralization rates, higher total N content, and altered AOA and AOB population sizes. Shoot, litter and root biomass were also higher in areas invaded by smooth brome. These changes corroborate the growing body of evidence that invasive species significantly alter soil nitrogen cycling, although the direction of change can depend on the identity of the invasive species (Ehrenfeld, 2003; Laungani and Knops, 2009; Liao et al., 2008). While this study found higher gross mineralization rates, other studies examining brome species found reduced, or negligible differences in mineralization rates (Evans et al., 2001; Fink and Wilson, 2011; Nosshi et al., 2007). These studies measured net, rather than gross mineralization, highlighting an important change that may not have been captured by examining net rates. Although the timing of this study may have been influential, the reported values in this study are similar to other studies (Accoe et al., 2004; Bedard-Haughn et al., 2006), including one study of Saskatchewan soils that reported peak N cycling rates during July (Bedard-Haughn et al., 2006). Also, as smooth brome is a C_3 grass similar to the native species at the field site, peak productivity (and potential effects on N cycling) would likely occur within a similar time frame.

Higher mineralization rates in smooth brome stands are likely being driven by the increased biomass being produced above and belowground by smooth brome. Litter is an important link between the plant and soil microbial community,

as the quantity and quality of litter produced by the plant community can affect the abundance and activity of the microbial community (Zak et al., 2003). Although the litter was of marginally poorer quality in brome stands, significantly more N is added to the soil given the greater litter biomass production. Incorporation of this additional litter N into the soil organic matter and the resulting higher total soil N, would provide additional substrate for the microbial community, resulting in stimulated gross mineralization rates (Booth et al., 2005; Zak et al., 2003). Many studies have reported higher litter quality in invasive species (Ehrenfeld, 2003; Liao et al., 2008) but similar to our observations, Nosshi et al. (2007) found no difference in litter quality between a range of C_3 grasses and smooth brome.

Although gross mineralization rates are higher in soils under smooth brome, it is likely that much of this additional mineral N is immobilized in the microbial biomass (Knops et al., 2002). Greater immobilization rates may explain why no increase in ambient ammonium or nitrate in invaded soils was observed, and why previous studies reported no change in net mineralization (Evans et al., 2001; Fink and Wilson, 2011; Nosshi et al., 2007). As the amount of nitrogen available to the plant community is dependent on immobilization rates (Knops et al., 2002), greater immobilization may prevent any net change in plant available nitrogen. Despite greater potential immobilization, smooth brome is able to produce greater biomass compared to native species. This suggests other mechanisms drive the productivity of this species. As smooth brome is a clonal plant, N transfers between ramets may be an important mechanism to allow the expansion and maintenance of smooth brome (Otfinowski and Kenkel, 2008). Greater root biomass in smooth brome soils

may also suggest that smooth brome has greater access to soil nutrients compared to the native plant community.

Despite higher mineralization rates, gross nitrification rates did not change. Nitrification rates were lower than mineralization rates in these soils, suggesting that the role of nitrification in this system may be limited. This result is supported by a review by Booth et al. (2005), which showed that nitrification is only an important fate for ammonium when mineralization rates are low. It is likely that the nitrifying community in this grassland is in competition with other soil microbes and with the plant community for ammonium, but neither the intensity of this competition or the effect of smooth brome invasion on this competition is known (Hodge et al., 2000; Kaye and Hart, 1997). In particular, if the higher gross mineralization rates are stimulating immobilization, very little ammonium may be available for nitrification.

Although no changes in gross nitrification rates were observed, AOA and AOB population sizes appeared to respond to smooth brome invasion. Similarly, a mesocosm study found higher AOB populations under exotic grass stands, but these soils also had higher nitrification rates (Hawkes et al., 2005). However, Hawkes et al. (2005) did not examine the AOA population, which was more abundant in the present study. AOA are typically more abundant than AOB (Leininger et al., 2006; Schleper, 2010), but it is unclear why AOA and AOB responded differently to smooth brome. Little is known about differences in the autecologies of these two functionally similar groups (Schleper, 2010; Taylor et al., 2012). However, changes in the root biomass or root exudate composition due to changes in plant community

structure may have differential impacts on these two groups (Lamb et al., 2011). To examine the potential relationship between root biomass and AOA/AOB copy numbers, an additional model was run to assess this relationship. There was a positive relationship between root biomass and AOA ($F_{1, 58}$=13.9,p<0.001), and AOB ($F_{1, 58}$=53.6,p<0.001), suggesting that AOA and AOB may be influenced by the root exudates released by smooth brome. However, as there was no change in gross nitrification rates and the effects of smooth brome were weak, this finding should be interpreted with caution. Additionally, the diversity of the AOA and AOB communities was not measured, which may also influence nitrification rates (Horz et al., 2004; Ma et al., 2008).

In summary, this study demonstrates that areas invaded by smooth brome have altered nitrogen cycling processes compared to native grassland. The hypothesis that soil conditions were altered prior to invasion cannot be ruled out, but this study shows that these conditions are at least maintained under smooth brome. Altered nitrogen cycling patterns at the invasion front of a clonal patch of smooth brome may facilitate invasion of this grass into a new host area. Future research examining nitrogen cycling rates across time or in newly invaded areas will provide a further understanding of initial invasion conditions and seasonal influences, as well as an indication of the long-term legacy effect of smooth brome.

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Chapter Preamble

This chapter identifies important consequences of smooth brome invasion to above and belowground communities in a Fescue Grassland. The study in Chapter Two demonstrated that smooth brome invaded areas had higher mineralization rates compared to native grassland, likely due to higher plant productivity and stimulated soil microbial activity. In the next chapter, the relationship between smooth brome and the soil bacterial community is investigated. Smooth brome shifted plant community composition, and plant richness and evenness were lower in invaded areas. However, soil bacterial richness and evenness increased with increasing smooth brome cover. Despite strong direct and indirect influences of smooth brome on soil organic carbon, total nitrogen and root biomass through increased plant biomass, none of these variables were important predictors of bacterial community composition. This suggests an alternative mechanism by which smooth brome influences soil bacterial community structure. Smooth brome was also associated with bacterial groups important for nitrogen cycling. These responses demonstrate how invasion, through a cascade of effects on the plant and soil communities can potentially alter important ecosystem services such as nitrogen cycling.

This chapter relates to the overall thesis because it addresses the objective of examining the influence of smooth brome on the soil ecosystem. This chapter examines how smooth brome, through changes in plant community composition and productivity, influences soil bacteria, an essential soil ecosystem component.

3 Direct effects of an altered plant community with smooth brome invasion on soil bacterial community structure and composition

3.1 Abstract

Plant and soil communities are tightly linked, but it is unknown how the invasion of an exotic plant and the resulting shifts in plant community diversity and productivity influence soil bacterial community richness and evenness. As soil bacteria are responsible for processes such as organic matter decomposition, changes to the bacterial community structure or composition may represent important changes to ecosystem processes. To investigate the relationship between invasive species and the soil bacterial community, 16S massively parallel sequencing was used to determine bacterial community richness, evenness and composition from soil collected from a smooth brome (*Bromus inermis* Leyss)-invaded grassland. As bacterial community richness and evenness increased with increasing smooth brome cover, structural equation modeling was used to tease out potential mechanisms for these results. Although it was hypothesized that soil organic carbon, total nitrogen and root biomass would be important predictors of bacterial community richness or evenness, none of these pathways were significant. These models did support, however, direct relationships between smooth brome shoot biomass and plant richness, suggesting an alternative, unknown mechanism between the plant and soil bacterial communities. Potential changes in root exudate carbon profiles or changes in food web dynamics may be this unknown mechanism. Increased smooth brome abundance was also associated with changes in the

abundance of bacteria important in nitrogen cycling. These responses highlight the important belowground consequences of smooth brome invasion that may have significant consequences for ecosystem functioning.

3.2 Introduction

Plant and soil communities are tightly linked (Hooper et al., 2000; van der Heijden et al., 2008; Wardle, 2002)**,** and changes in the plant community induced by plant invasion have important ecosystem consequences. Mechanisms of plant control of the soil bacterial community include litter quality and quantity (Strickland et al., 2009), and root exudate composition (Eilers et al., 2010; Haichar et al., 2008; Wardle, 2002). Plant community control on bacterial community structure can be variable as increased plant richness has been associated with greater microbial biomass and altered composition in some studies (Bartelt-Ryser et al., 2005; Lamb et al., 2011b; Zak et al., 2003), while others found weak or no influence of plant community structure (Cruz-Martinez et al., 2009; Kielak et al., 2008; Porazinska et al., 2003). New evidence suggests soil bacteria may respond indirectly to plant species richness through increased plant biomass (De Deyn et al., 2011). This lack of clear response may be due to the weak taxonomic resolution (e.g. PLFA analysis) at which the bacterial communities were examined, potentially masking any finer scale responses. With the growing evidence that bacterial species richness (Bell et al., 2005; Jiang, 2007) and evenness (Wittebolle et al., 2009) are important indicators of ecological functioning, a clear understanding of the influence of plant community composition on these variables is lacking.

The dramatic shift in plant diversity and productivity associated with exotic plant invasion provides a natural experiment to test the relationship between plant and bacterial communities. Many invasive species strongly interact with the soil bacterial community (Inderjit and van der Putten, 2010; Klironomos, 2002; Kourtev et al., 2002; van der Putten et al., 2007; Wolfe and Klironomos, 2005) including some cases of positive feedbacks back to the invasive plant (Jordan et al., 2008; Klironomos, 2002). As invasive species typically reduce plant richness and evenness (Vilà et al., 2011) and increase productivity (Liao et al., 2008), invasion may result in more, but less diverse plant inputs to the soil. This altered resource environment is important to bacterial community composition (Eilers et al., 2010; Jones et al., 2009; Ramirez et al., 2010). However, legacy effects (Elgersma et al., 2011) and food web dynamics (Belnap and Phillips, 2001) add complexity to the response of a bacterial community to a changing resource environment. Although manipulative studies can be used to isolate individual relationships (e.g. Hawkes et al., 2005), in-situ field experiments that explicitly incorporate ecosystem structure provide the most realistic representation of the invasive plant-soil bacteria relationship. However, incorporation of this complexity increases the difficulty of disentangling feedbacks between plants, bacteria, and soil properties. Structural equation modeling is one approach that can be used to untangle complex responses to invasive species (Grace, 2006; Lamb et al., 2011a; Lamb et al., 2011b), as it can be used to determine the importance of direct and indirect relationships in a network of interacting variables.

Despite the important contribution of soil bacterial communities to ecosystem services (Hooper et al., 2000; Wardle, 2002), very little is known about individual ecological roles within this highly diverse group (Fierer et al., 2007; Fierer and Lennon, 2011; Torsvik et al., 2002). Exceptions to this knowledge gap are limited to bacteria with well-defined ecological roles, such as nitrifying or N-fixing bacteria, although some bacterial phyla respond to soil carbon availability (Fierer et al., 2007). There is likely some degree of functional redundancy within the bacterial community (Chapin et al., 1997), however the historical adaptation of a bacterial community to its resource environment may be important in determining ecosystem function rates (Allison and Martiny, 2008; Elgersma et al., 2011; Strickland et al., 2009). Changes to this resource environment (such as those induced by a change in the dominant plant species) may have important consequences for ecosystem function (Allison and Martiny, 2008; Elgersma et al., 2011; Strickland et al., 2009).

In this study the influence of the invasive grass smooth brome (*Bromus inermis* Leyss) on the structure and composition of the soil bacterial community is examined using Ion Torrent sequencing. This analysis provided a detailed, taxonomic based assessment of the bacterial community. First, the hypothesis that smooth brome would influence bacterial species richness and evenness was examined. Secondly, we examined the hypothesis that the relationship between plant and soil bacterial communities was mediated through changes in soil properties and root biomass (see Chapter 2) using structural equation modeling (SEM). Thirdly, the complex relationships between the abundance of different

bacterial groups and changing plant community composition was explored using non-metric multidimensional scaling (NMS).

3.3 Materials and Methods

3.3.1 Field Site

The field site is a 14.6 ha native fescue prairie undergoing invasion by smooth brome, approximately 120 km south of Saskatoon, SK., Canada (51°12' N 107°17' W) (Figure 3.1). The site is near the border of the Moist Mixed and Mixed Prairie ecoregions and within the Orthic Dark Brown Chernozemic soil order (Agriculture and Agri-Food Canada, 2010). A total of 65 plant species were found at the site. Dominant native grasses included *Festuca hallii*, and several species of *Hesperostipa, Elymus and Pascopyrum.* A variety of forbs were also abundant. The landscape consisted of rolling hills, with upland species such as *Koeleria macrantha* and *Bouteloua gracilis* occurring on hilltops, and more shrubby species (e.g. *Symphoriocarpus occidentalis*) occurring in lower areas. Smooth brome is invading the site from disturbed edges (primarily roads), and many invaded patches can be found in the interior of the site (Figure 3.1). No management practices are present on the site and have not been for at least 27 years (Jim Romo, personal communication). Average yearly temperature is 3.5˚C and the average yearly precipitation for this area is 376.9 mm (Rock Point weather station, ~7 km from field site, Environment Canada, 2012).

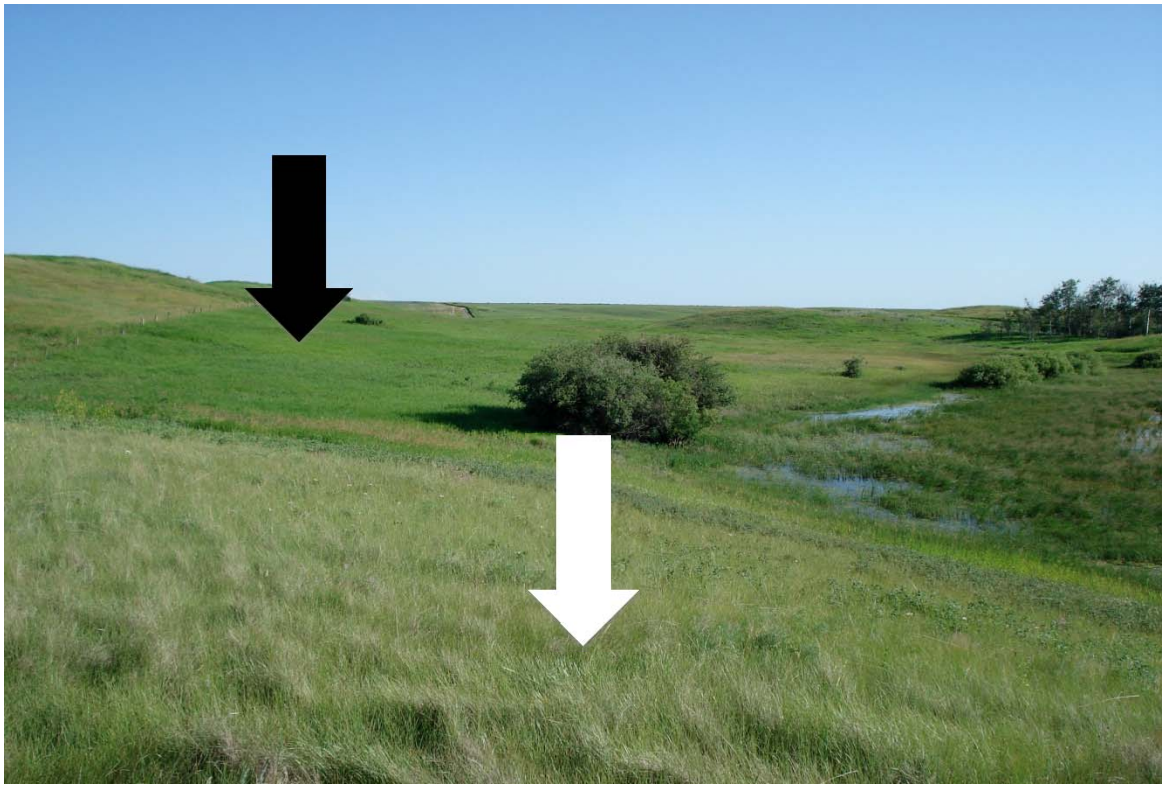

Figure 3.1 Photo of field site showing the distribution of smooth brome in the landscape. Foreground of photograph is native grassland (white arrow), and in the background (black arrow) is a large patch of smooth brome.

3.3.2 Sample Collection

A stratified random sampling design was used to collect plant and soil samples from a broad range of smooth brome cover classes. A total of 60 locations with 15 samples in each of four categories of aboveground smooth brome abundance (0%, >0-50%, 51-85% and >85%) were sampled. Random sampling locations were determined using the random point generator in ArcMap (Esri, Redlands, CA., U.S.A.). At each location, plant species cover was assessed and grass, forb, shrub, and litter biomass was collected within a 50 x 50 cm quadrat. Smooth brome biomass was collected separately from other grass species. Biomass samples

were dried for 2 days at 60˚C and weighed. To determine litter C:N ratio, a subsample of dried litter was ground and analyzed on a Leco AutoAnalyzer (Leco Corp., St. Joseph, MI., U.S.A) for total carbon and nitrogen.

After plant biomass collection, we measured the depth of the A horizon using changes in soil color and texture (Agriculture and Agri-Food Canada, 1998). Using an AMS soil corer (AMS, Inc., American Falls, ID., U.S.A.), two soil cores (5 x 5cm) were extracted from each of the A and B horizons. The two soil cores within each horizon were combined, resulting in one composite soil core from each horizon at each location. These samples were frozen at -20˚C. From each soil core, roots were carefully picked out and weighed. Fresh weights were taken as the roots were intended to be used in a separate study of root distributions. Soil organic C was determined using a Leco Carbonator (Leco Corp., St. Joseph, MI, U.S.A.), and soil total nitrogen was determined using a Leco AutoAnalyzer (Leco Corp., St. Joseph, MI., U.S.A).

3.3.3 Bacterial diversity assay

Bacterial diversity was assessed using Ion Torrent massively parallel sequencing (Life Technologies) of the 16S rRNA gene region (Fierer and Lennon, 2011; Hirsch et al., 2010). DNA was extracted from 0.5 g of 2 mm sieved soil using the Ultraclean Soil DNA extraction kit (MoBio, Carlsbad, CA., U.S.A.), and stored at -20 ˚C until use. DNA concentration was determined using a UV-Vis spectrophotometer (Nanodrop 2000, ThermoScientific, Wilmington, Del. U.S.A.). We used the universal 16S rRNA bacterial primer set 515F/806R, which amplifies a 291 bp fragment near the bacterial v4 region (Earth Microbiome Project, 2011). Primers

contained an Ion Torrent adapter and a unique barcode sequence for sample pooling. Samples were amplified in triplicate using a 25 µl reaction mix containing: 18 µl Platinum Blue Supermix (Invitrogen), 0.2 µM reverse and barcoded forward primer, and 5 µl DNA (50 ng DNA). Thermocycling conditions were as follows: 94 °C for 5 min, 20 cycles of 94 °C for 30 sec, 60 °C -0.5 °C for 1 min, and 72 °C for 1 min, followed by 10 cycles of 94 °C for 30 sec, 55 °C for 1 min, and 72 °C for 1 min, and a final extension for 7 min at 72 °C. PCR products were checked on a gel, and sample replicates were pooled and purified using a QiaQuick gel extraction kit (Qiagen). We ensured sufficient purified product was generated using a gel and quantification ladder (Invitrogen Low Mass Ladder). Six samples were not sequenced as insufficient product for sequencing could be amplified. Ion Torrent sequencing was completed by Contango Strategies (Saskatoon, SK., Canada). Sample concentrations were determined using the Qubit 2.0 Fluorometer (Life Technologies) and pooled in equal molar amounts. Following pooling, the samples were sequenced according to the Ion PGM 200 Sequencing Kit v2 (Life Technologies).

Samples from the Ion Torrent platform were processed and analyzed using the mothur software package (Schloss et al., 2009). Sequence files (fasta) and quality scores for the base calls (qual) were extracted from the sff files provided by Contango. Reads were interrogated for indicators of poor quality including short length (<80bp), homopolymerism (>8bp homopolymers), lack of homology to the barcode sequences, a lack of homology of more than 2bp difference to the primer sequence and an overall average quality score from the qual file of less than 25 (shown to be indicative of poor quality sequence replication) (Huse et al., 2007).

Any reads that fell into these criteria were removed from the dataset. Subsequently the reads were grouped behind a seed sequence if they were identical to reduce redundancy in the dataset and improve processing capability. The 'unique' reads were aligned using a NAST algorithm against the SILVA seed database containing information that incorporates secondary structure of the 16S rRNA molecule as this is shown to improve operational taxonomic unit (OTU) assignment (Caporaso et al., 2010; Pruesse et al., 2007; Schloss, 2012; Schloss and Westcott, 2011). Aligned seed sequences were then trimmed to the same length and subject to detection of chimaeric artifacts using the UCHIME algorithm via the mothur program (Edgar et al., 2011).

The remaining high quality chimaera free reads were subsampled to 2550 reads to normalize the read distribution and sampling effort across all the samples. A distance matrix was created using the subsampled dataset and OTUs were clustered at 97% sequence similarity. Those OTUs occurring only once in the entire dataset (singletons) were removed as an additional precautionary quality control and an OTU by sample matrix was produced to allow comparison of the samples. Taxonomic assignment of OTUs was achieved by BLAST comparison of the seed sequences against the Greengenes 2011 database (McDonald et al., 2012) by trimming the Greengenes sequences to the same region as the seed sequences to improve assignment (Werner et al., 2012). Community evenness was calculated using Evar (Smith and Wilson, 1996) in the R package (R Core Development Team, 2012), and species richness was determined as the total number of species (OTUs) present in each sample.

3.3.4 Statistical analysis

The relationship between smooth brome and bacterial species richness and evenness was examined using linear mixed models. Smooth brome shoot biomass was used as an indicator of smooth brome abundance and was used along with horizon as explanatory variables. "Plot" was included as a random factor. Mixed models were run in R (R Core Development Team, 2012) using the nlme library (Pinheiro et al., 2012). Fitted vs. residual and qq plots were used to ensure appropriate model fit.

This initial assessment showed significant effects of smooth brome on bacterial community richness and evenness. To investigate the potential mechanisms underlying these effects, a multi-group structural equation model (SEM) was fit using plant productivity and soil composition data as observed variables. SEM was chosen because it allowed for separation and testing of the direct and indirect relationships between intercorrelated variables (Grace, 2006; Lamb et al., 2011a). The first step in SEM is to develop an initial path model based on prior theoretical knowledge about the system. The second step is to test for fit between the implied covariance structure of the theoretical model and the actual covariance structure of the data. Initial fit between the model and data provides strong support for the theoretical relationships being tested. A multi-group model is appropriate for this dataset as we have two subsets of data (A and B horizon) collected from the same sample points. In a multi-group SEM, models are initially constrained so that path coefficients are equal between groups. These constraints can then be progressively released to improve model fit. A difference in path

coefficients between horizons indicates a significant difference between horizons in the biological process represented by that path.

The initial SEM model was developed to examine the influence of changing plant shoot community composition on soil bacterial community structure (Figure 3.2). Smooth brome shoot biomass and plant species richness were used as indicators of plant community composition. We hypothesized that brome may influence plant richness directly, or indirectly through changes in litter biomass. As plant species richness may also be influenced by site productivity, we included a direct relationship between A horizon depth (an indicator of long-term site productivity) and native plant species richness. As different plant species produce litter of differing quality and composition (Cornelissen, 1996; Wardle, 2002) we included direct relationships from brome biomass and native species richness to litter C:N ratio, a measure of litter quality. As leaf litter and root decomposition are important sources of organic carbon and nitrogen, we included direct relationships from litter quality and quantity and root biomass to soil organic carbon and total nitrogen. Soil organic carbon and total nitrogen were used as predictors of bacterial community richness and evenness, as resource availability is known to influence bacterial community composition (Drenovsky et al., 2004; Fierer et al., 2003). Changes in root biomass may also influence bacterial community structure as plant roots and soil bacteria are strongly linked (Wardle et al., 2004). We also included bivariate (non-directed) relationships between soil organic carbon and total nitrogen. Mean values for all variables used in the models are given in Table 3.1.

Figure 3.2 Initial structural equation model. Single-headed arrows represented directed relationships and double-headed arrows represent bivariate relationships (undirected).

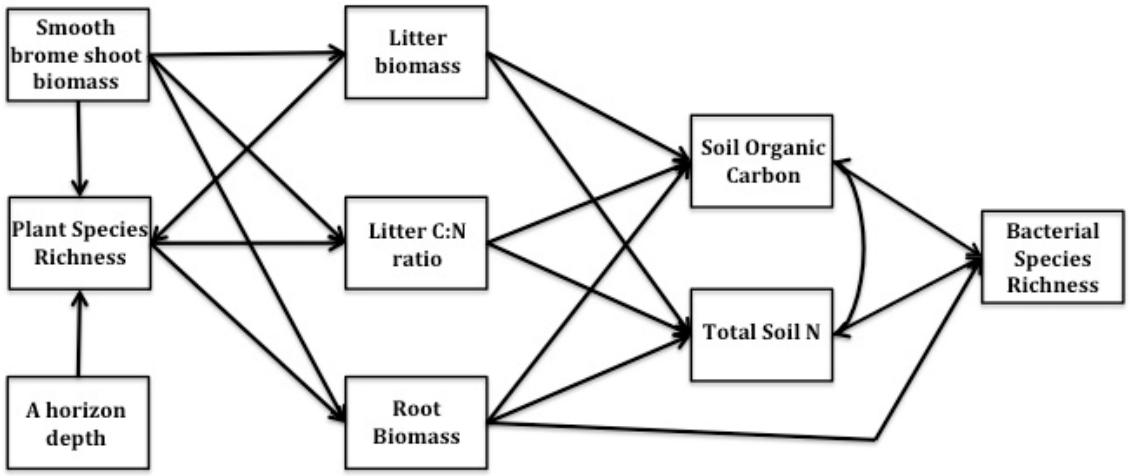

Table 3.1 Mean and standard deviation for all variables included in structural equation modeling analysis. For horizon-level data, asterisks indicate differences in mean values between horizons (t-test, $p<0.001$).

Variable	Mean ± standard deviation
Smooth brome shoot biomass (g/m^2)	265 ± 211.2
Plant richness	11 ± 3.55
A horizon depth (cm)	12 ± 4.16
Litter biomass (g/m^2)	336 ± 160.0
Litter C:N ratio	23.6 ± 5.60
Root biomass (g/m^2)	
A Horizon	1612 ± 749.6**
B Horizon	433 ± 302.6
Soil organic carbon (%)	
A Horizon	6.0 ± 1.90**
B Horizon	2.5 ± 0.787
Total soil nitrogen (%)	
A Horizon	0.61 ± 0.168**
B Horizon	0.27 ± 0.0922
Bacterial species richness	
A Horizon	616 ± 122.8**
B Horizon	508 ±118.6
Bacterial community evenness	
A Horizon	0.63 ± 0.0490**
B Horizon	0.59 ± 0.0534

Prior to fitting the SEM, relationships were checked for linearity using general linear models that included a quadratic term. Significant quadratic terms were found for relationships involving root biomass; these relationships were linearized by log transforming root biomass. Smooth brome shoot biomass, litter biomass, and bacterial species richness were divided by $1.0x10^4$ to equalize variances. The SEM models were fit using the lavaan library in R (Rosseel et al., 2012). The SEM model was built step-wise, first fitting a single SEM model with only bacterial species richness. As this model had adequate fit, it was fit as a multi-group model with all parameters constrained to be equal. This model did not have adequate fit (χ^2_{55}=99.0, p<0.001), but through sequential release of parameter constraints with high standardized residuals, it reached adequate fit (χ_{53}^2=62.2, p=0.180). A second model was fit with bacterial community evenness replacing richness. The initial single evenness model had adequate fit, but the multi-group model did not initially have good fit (χ_{55}^2=100.0, p<0.001). Through release of parameters, it reached adequate fit (χ_{53}^2=62.2, p=0.180). Both the richness and evenness models showed that none of the variables predicting bacterial richness or evenness were important. To confirm that smooth brome was in fact influencing these variables, direct relationships were added from brome shoot biomass and plant richness to bacterial richness and evenness. These *ad hoc* pathways were added to represent an unknown mechanism rather than a direct theoretical relationship. Increased χ^2 values and decreased Akaike's Information Criterion (AIC) values were used to determine if these added direct relationships improved model fit (Akaike, 1974).

The relationships between plant and bacterial community composition were examined using non-metric multidimensional scaling (NMS). NMS was used as it is robust for ecological, non-normal datasets (McCune and Grace, 2002). Plant community data were ordinated using the Sorensen (Bray-Curtis) distance metric in PC-Ord 5 (Kruskal, 1964; Mather, 1976; McCune and Mefford, 2006). Separate ordinations were run for each horizon due to different missing sample points in the A and B horizon bacterial datasets. Ordinations were completed using random starting configurations and 50 runs with real data. For both horizons, a two dimensional solution was chosen (Stress $_{\text{A Horizon}}$=16.6, Stress $_{\text{B Horizon}}$=15.9), and the Monte Carlo test was significant (p=0.0196). The final solution was based on 200 iterations. Both ordinations were rotated graphically so that Axis 1 was most correlated with smooth brome abundance. To explore the relationships between the plant and bacterial community, a joint plot (r^2>0.1) of bacterial abundance aggregated at the phylum and order level was overlaid to examine broad relationships between the plant and bacterial community. Some OTUs could not be classified to species or were classified to unnamed taxonomic groups, and therefore OTUs were named to known taxonomic level. OTUs that were classified to a taxonomic group with certainty lower than 75% were changed to unclassified for that level of taxonomic resolution.

3.4 Results

3.4.1 Influence of smooth brome on bacterial richness and evenness

Bacterial species richness increased in both the A and B horizon with increasing smooth brome biomass (Figure 3.3). Species richness (R) was higher in

the A horizon (R=616±123) compared to the B (R=508±119) ($F_{1, 58}$=24.6, p<0.001) (Figure 3). The horizon by brome interaction term was not significant ($F_{1, 52}$=2.80, p=0.100). Bacterial community evenness increased with increasing smooth brome biomass in both the A and B horizons (Figure 3.3). Evenness was higher in the A (E_{var}=0.63±0.05) than the B horizon (E_{var}=0.59±0.05) ($F_{1, 58}$=20.4, p<0.001) (Figure 3.3). The horizon by brome interaction term was not significant for evenness ($F_{1,52}$=1.78, p=0.190). Rather than large changes in the abundance of a small number of individual bacterial species, it appears that many rare species in native grassland soils increased in abundance in soils invaded by smooth brome (Figure 3.4).

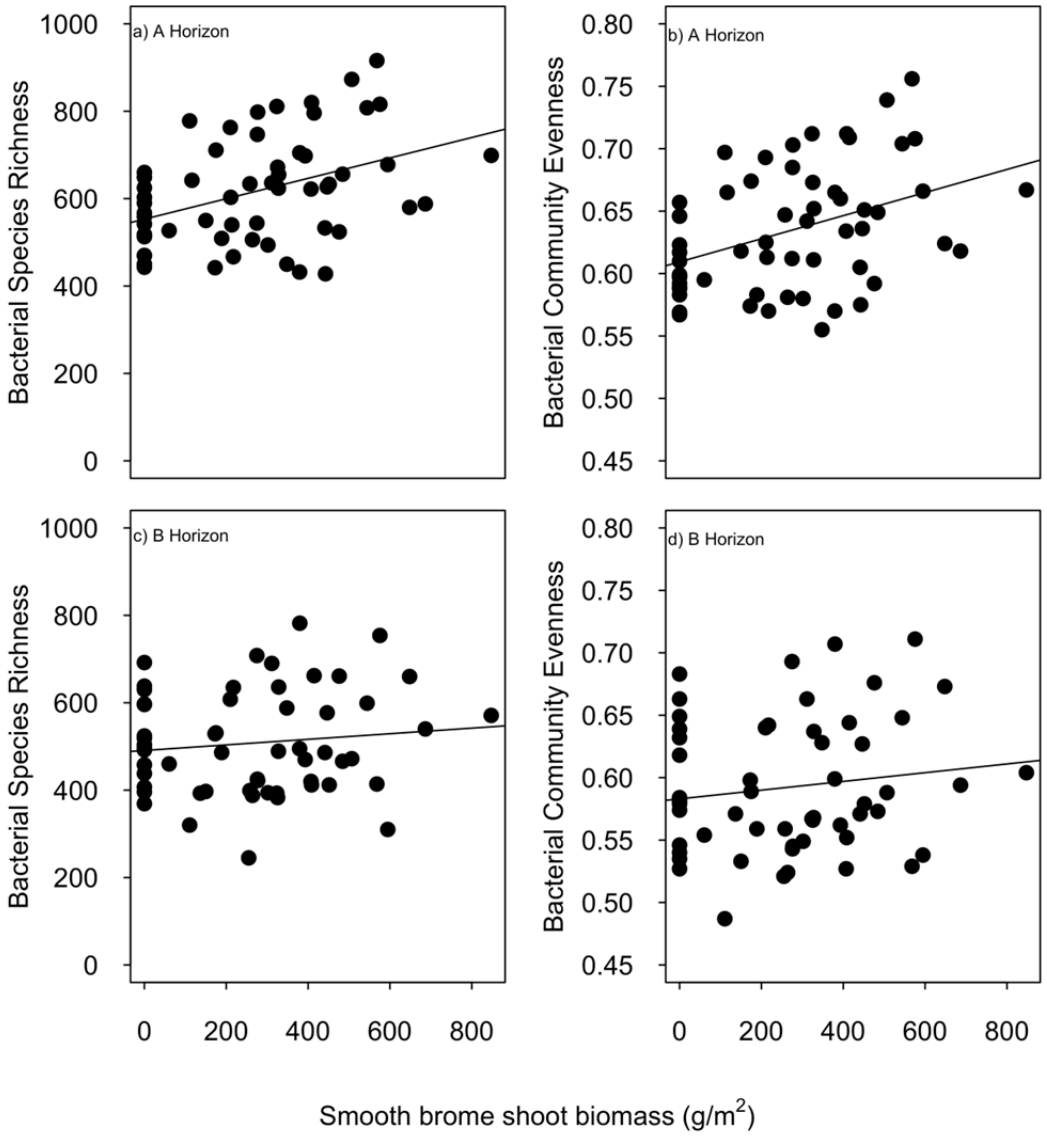

Figure 3.3 Bacterial species richness ($F_{1,58}$=8.64, p=0.005) and evenness ($F_{1,58}$=8.81, p=0.005) increased significantly with increasing smooth brome cover: a) and b) are A horizon data, and c) and d) are B horizon relationships. Regression lines are significant for species richness in the A (*Species richness*=0.24(0.07)x +553(24.4), r^2=0.185), and B horizon (*Species richness*= 0.24(0.07)x +490(34.7), r^2=0.005). Regression lines were also significant for community evenness in the A horizon

(*Community evenness*=0.000093(0.000030)x +0.61(0.0104), r^2=-0.183) and the B horizon (*Community evenness*=0.000093(0.000030)x +0.58(0.0105), r^2=0.012).

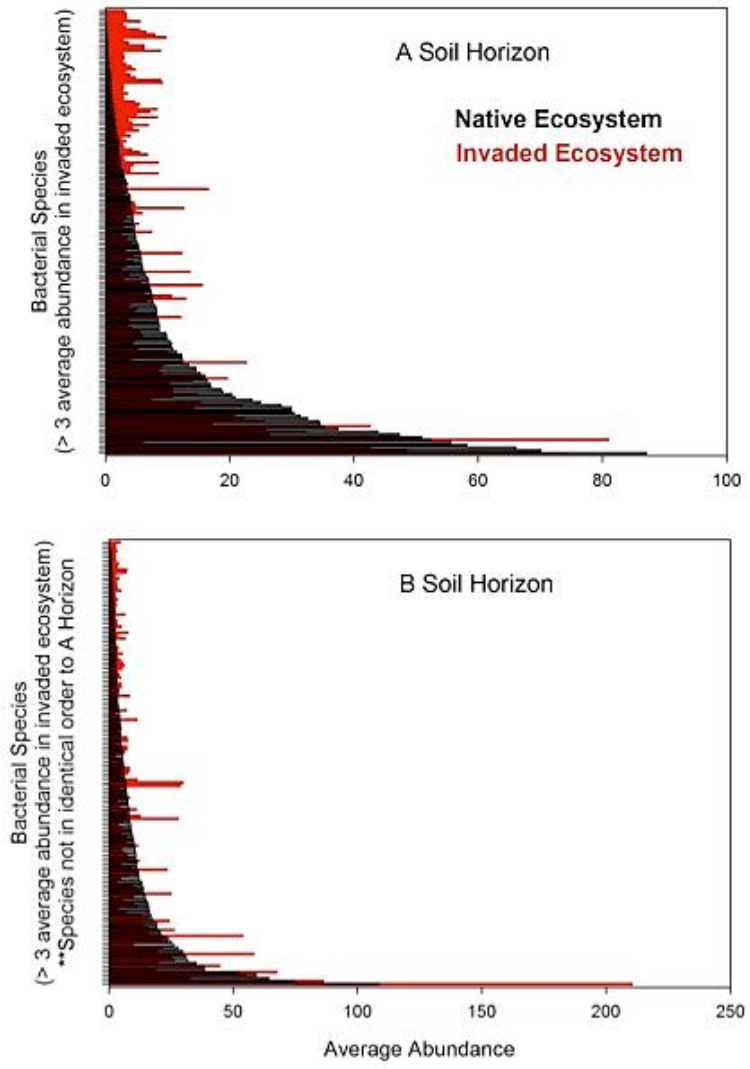

Figure 3.4 Average abundance of bacterial species ordered from least to most abundant in the native grassland soil. Black bars represent the abundance of each bacterial species in the native grassland soils, and red bars represent the abundance of the same bacterial species from soils with >85% smooth brome invasion. Note

that the order of bacterial species between horizons is not the same, and that only bacterial species that occurred more than three times are included in this graph.

3.4.2 Mechanisms behind altered bacterial community structure

Smooth brome increased litter and root biomass, and the increase in litter biomass reduced plant species richness (Table 3.2, Figure 3.5, Figure 3.6, Appendix Tables 5.1-4). There was no significant direct relationship between smooth brome and litter C:N ratio, but smooth brome indirectly influenced litter quality via changes in plant species richness. Litter C:N ratio did not influence soil organic carbon or total nitrogen, but increased litter and root biomass resulted in higher soil organic carbon and total nitrogen. Although smooth brome influenced soil organic carbon, total soil nitrogen, and root biomass through both direct and indirect effects, none of these variables were important predictors of bacterial species richness or evenness. The *ad-hoc* additions of direct relationships between smooth brome shoot biomass and plant species richness improved fit for both models (Table 3.2), and pathways from smooth brome biomass and plant species richness were significant (Figure 3.4, Appendix Tables 5.3, 5.4). Consistent with previous results, smooth brome shoot biomass increased bacterial richness and evenness, while plant species richness decreased richness and evenness.

Table 3.2 Chi-squared values (χ^2) , degrees of freedom (df), p-values, Comparative Fit Index (CFI), Standardized Root Mean Square Residuals (SRMR) and Akaike's Information Criterion (AIC) for all SEM models.

	χ^2	df	p value	CFI	SRMR	AIC
Bacterial richness	62.2	53	0.180	0.978	0.108	184.0
Bacterial evenness	62.7	52	0.146	0.974	0.107	515.7
Bacterial richness with direct pathway from plant community variables	51.4	51	0.460	0.999	0.092	177.1
Bacterial evenness with direct pathway from plant community variables	50.0	51	0.513	1.00	0.090	505.1

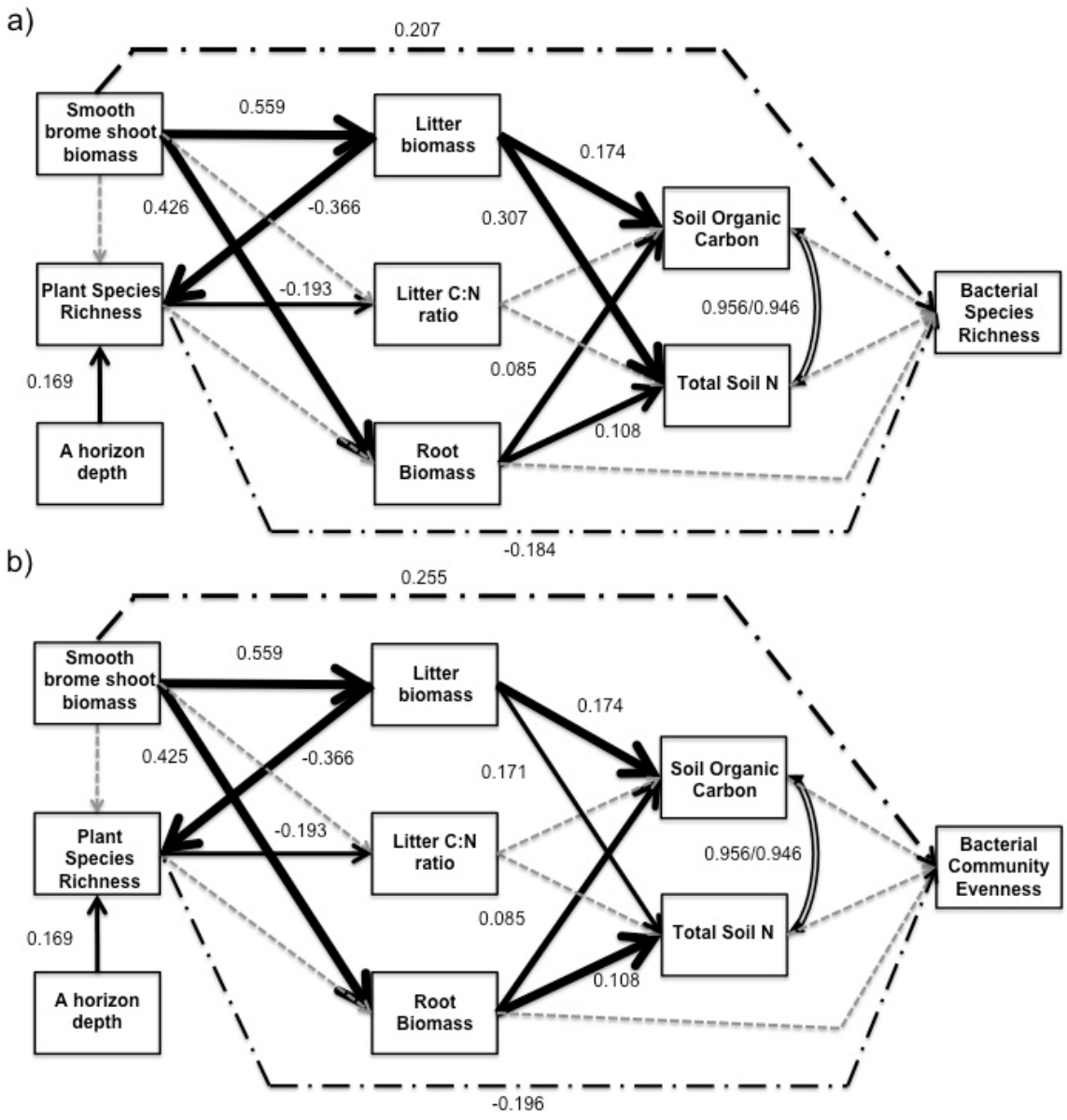

Figure 3.5 Multi-group structural equation models of smooth brome interactions with a) bacterial species richness and b) bacterial community evenness. Solid arrows represent significant relationships ($p<0.05$), and the thickness of the arrow indicates degree of significance. Grey dotted lines represent non-significant relationships. Standardized path coefficients are shown next to significant pathways. A double line represents cases where parameters differed between

horizons, and two path coefficients are shown. The first one is the coefficient for the A horizon, and the second for the B horizon. Additional direct relationships from smooth brome shoot biomass and plant species richness are shown as black dashed (— · —) lines with their respective standardized path coefficients

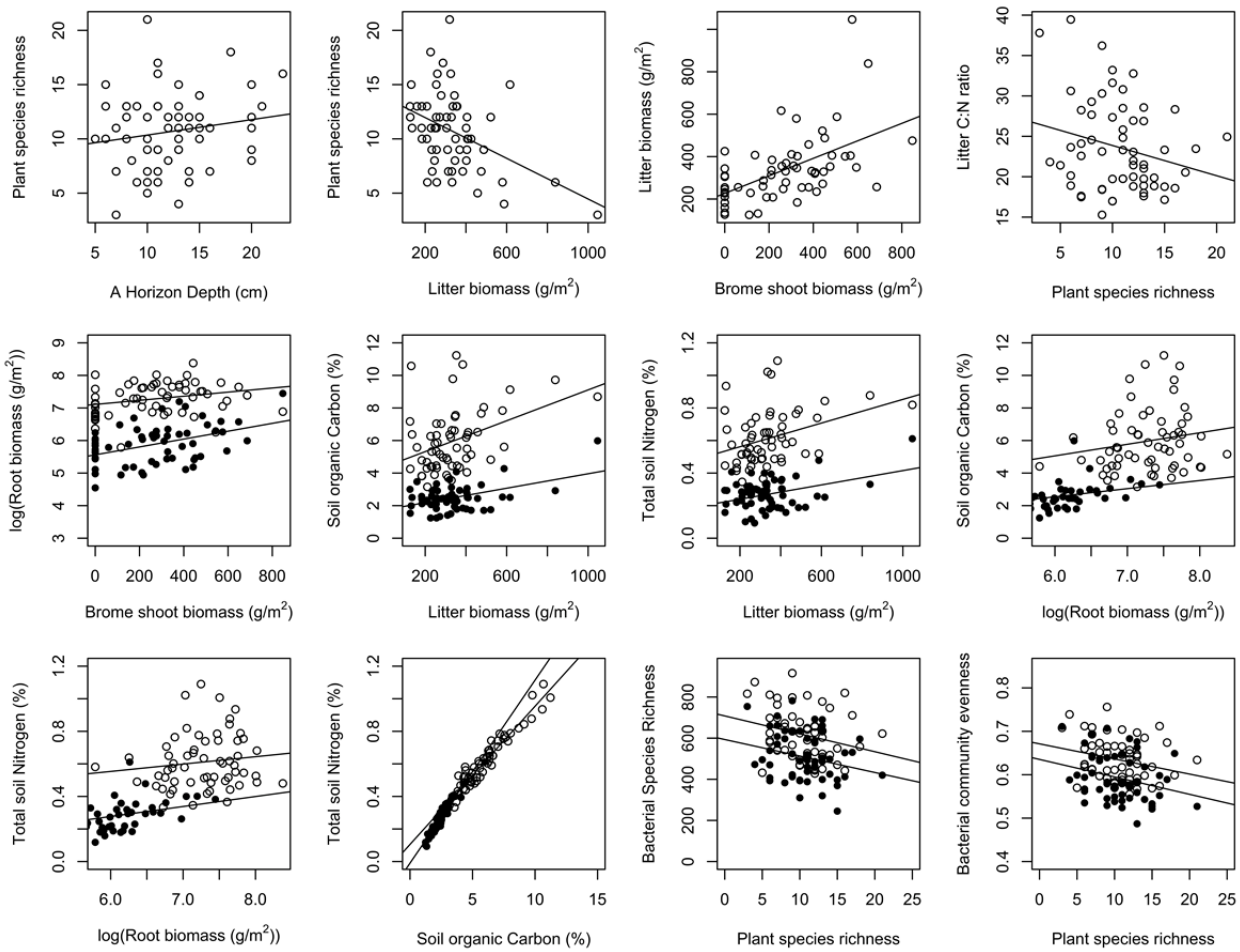

Figure 3.6 Bivariate plots and regression lines for all significant relationships in the SEM analysis. For variables with A and B horizon data, open circles represent A horizon data, closed circles are B horizon data.

3.4.3 Relationship between plant community composition and bacterial groups

There was a strong shift in plant species composition with increasing smooth brome cover (Figure 3.7). The cumulative proportion of variance explained for the A horizon over two axes was 0.815 (Stress=16.6), and 0.823 for the B horizon (Stress=15.9). For both ordinations, Axis 1 was highly correlated with smooth brome cover ($r^2_{A\ Horizon}$=0.786, $r^2_{B\ Horizon}$=0.817), and Axis 2 was most highly correlated with the abundance of the native grasses *Festuca hallii* ($r^2_{A\ Horizon}$=0.421, $r^2_{B\ Horizon}$=0.308) and *Pascopyrum smithii* ($r^2_{A\ Horizon}$=0.139, $r^2_{B\ Horizon}$=0.211) (Appendix Tables 5.5, 5.6).

Overlaid joint plots of bacterial phyla and orders showed the abundance of several bacterial groups was associated with plant community composition (Figure 3.7, Appendix Tables 5.7-10). In the A horizon, six phyla were associated with changes in plant community composition (Figure 3.7a). The candidate phylum CCM11b (r=-0.503, r^2=0.253), a group with unknown ecological function, and *Nitrospirae* (r=-0.421, r^2=0.178), an important group of nitrite-oxidizing bacteria, were most strongly negatively associated with smooth brome. Candidate phylum ZB2 and *Cyanobacteria* were also negatively correlated with smooth brome cover (r^2 > 0.100). Only one group of unclassified bacteria was positively associated with smooth brome cover, and *Firmicutes* was positively associated with native grass abundance.

Several bacterial orders were associated with changing plant community composition in the A horizon (Figure 3.7b). The unnamed group within the CCM11b

candidate phylum (r=-0.503, r^2=0.253) and *Nitrospirales* (*Nitrospirae*) (r=-0.421, r^2=0.178) showed the greatest negative association with smooth brome cover. Several other orders were negatively (*Rhodocyclales* (*Proteobacteria*), Unnamed candidate phylum ZB2 order, *Chlorophyta* (*Cyanobacteria*)), and *Desulfurellales* (*Proteobacteria*)), and positively (candidate order A4b (*Chloroflexi*), *Chloroflexales* (*Chloroflexi*), candidate order B07_WMSP1 (*Chloroflexi*), *Enterobacteriales* (*Proteobacteria*), an unnamed bacterial order and *Chromatiales* (*Proteobacteria*)) associated with smooth brome cover (ordered in descending r^2). Bacterial orders also responded to native grass cover. *Bacillales* (*Firmicutes*), an unnamed order in the candidate GN07 class (candidate phylum GN02), and an unnamed order in the *Epsilonproteobacteria* (*Proteobacteria*) class were negatively associated with native grass cover, while *Legionellales* (*Proteobacteria*) and *Verrucomicrobiales* (*Verrucomicrobia*) were positively associated (Figure 3.7b).

In the B horizon, the Phylum *Chlamydiae* was negatively associated with both smooth brome cover (r=-0.318, r^2=0.101), and native grass cover (r=-0.341, r^2=0.116), and the candidate phyla CCM11b and GAL15 were negatively associated only with native grass cover (Figure 3.7c). Two bacterial orders of significance for N cycling were associated with smooth brome cover. *Nitrosomonadales* (*Proteobacteria*) was negatively associated with smooth brome cover (r=-0.318, r^2=0.101), while *Rhizobiales* (*Proteobacteria*) was positively associated (r=0.316, r^2=0.100). An unclassified *Proteobacteria* group and *Chlamydiales* (*Chlamydiae*) were also negatively associated with smooth brome cover (Figure 3.7d). Native grass cover was negatively associated with the orders *Chlamydiales* (*Chlamydiae*),

and *Euzebiales* (*Actinobacteria*) and positively associated with *Elusimicrobiales* (*Elusimicrobia*), unnamed groups in the *Epsilonproteobacteria* (*Proteobacteria*) and *Kueneniae* (*Planctomycetes*) classes, and an unnamed order in the CCM11b candidate phylum (Figure 3.7d) (ordered in descending r^2 values).

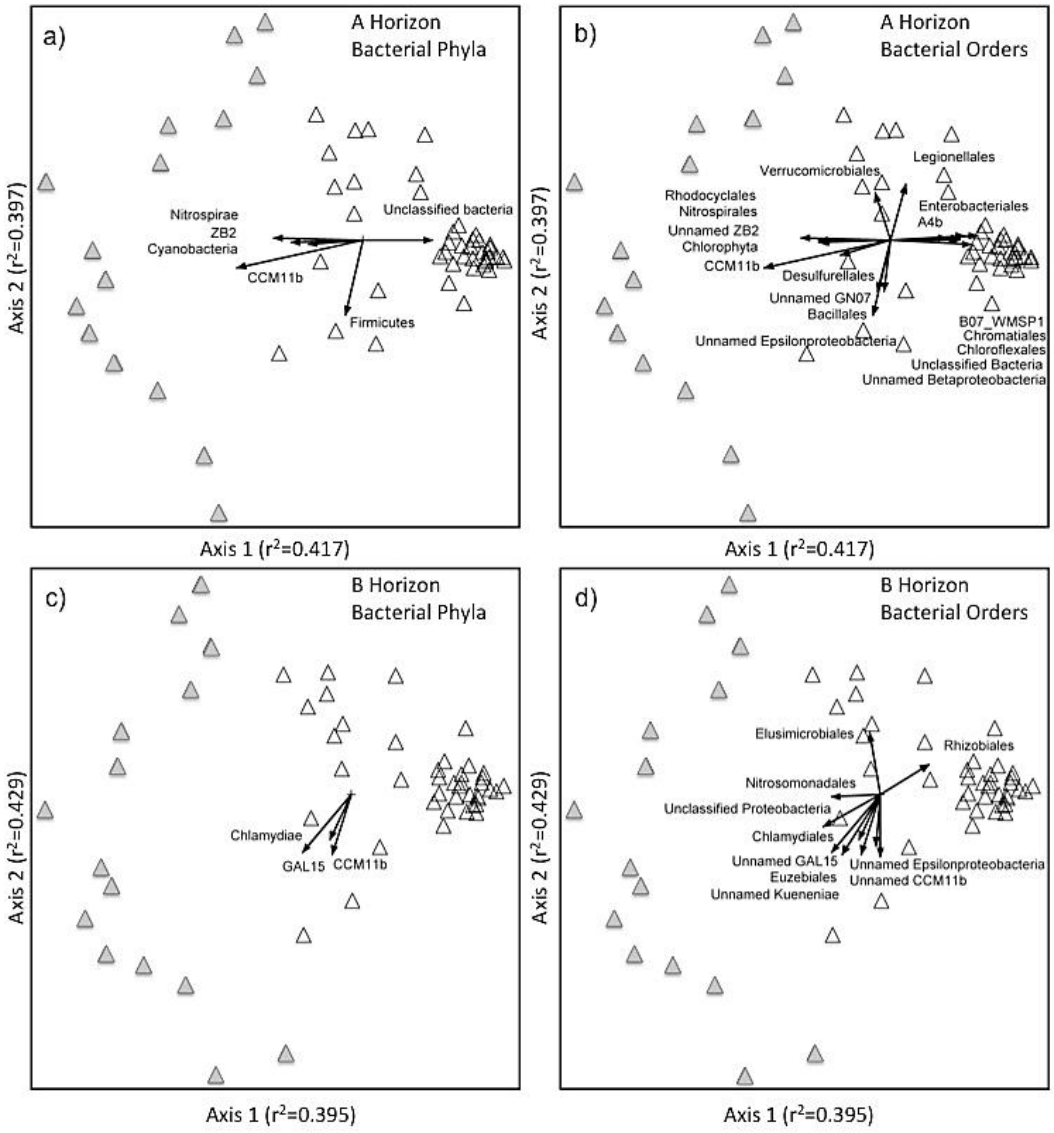

Figure 3.7 Nonmetric multidimensional scaling plot of plant community composition. Separate ordinations were completed for each horizon ($Stress_{A\ Horizon}$=16.6, $Stress_{B\ Horizon}$=15.9). Panels a) and b) are A horizon ordinations and c) and d) are B horizon ordinations. Axis 1 was highly correlated with smooth brome cover, and Axis 2 with native grass abundance. Gray-filled symbols represent native grassland plots, while open symbols represent plots where smooth brome was present. Bacterial data was aggregated at the phylum and order level, and overlaid

in joint plots (r^2>0.1); panels a) and c) are bacterial phyla overlays, and b) and d) are bacterial order overlays.

3.5 Discussion

Smooth brome invasion increased soil bacterial richness and evenness and was negatively associated with bacteria important for soil nitrogen cycling. Previous studies have documented effects of invasive species on soil microbial communities (Batten et al., 2006; Hawkes et al., 2005; Inderjit and van der Putten, 2010; Jordan et al., 2008; Klironomos, 2002; Kourtev et al., 2002; van der Putten et al., 2007); however, this study is one of the first to attempt to separate the complex mechanisms by which an invasive plant can influence the soil bacterial community. Smooth brome invasion was associated with a dramatic shift in plant community composition and reduced plant species richness. Contrary to changes in the plant community, bacterial species richness and evenness were higher in areas invaded by smooth brome. Although smooth brome increased soil organic carbon, total nitrogen and root biomass, these variables were not important predictors of bacterial richness or evenness. We were also able to associate the shift in plant community composition with invasion to changes in several important groups of bacteria. Notably, smooth brome was associated with declines in *Nitrospirae* and the *Nitrosomonadales* order, and increases in *Rhizobiales*.

Bacterial species richness increased with increasing smooth brome abundance and decreasing plant species richness in this study. This is an unexpected result as it was hypothesized that there would be a positive relationship between plant and bacterial richness. Individual plant species release a unique combination of root exudates that have a strong effect on rhizosphere bacterial communities (Bais et al., 2006; Haichar et al., 2008). Therefore, in more diverse

plant communities, root exudates should be more diverse and support greater bacterial diversity (Berg and Smalla, 2009; Coleman and Whitman, 2005; Hooper et al., 2000; Kowalchuk et al., 2002). The increase in bacterial species richness is not likely due to increased absolute numbers of species per se; rather, it is likely a consequence of changes in the relative abundance of bacteria species. As rare bacterial species became more abundant in smooth brome invaded soils, the bacterial community became relatively more even compared to the native grassland bacterial community. Increased abundance of rare species likely improved the probability of these species being detected, resulting in greater species richness. Altered abundance of rare species is also the primary driver of higher bacterial evenness in invaded soils, rather than significant decreases in the dominant species. This is demonstrated by the lack of, or at least very weak, associations of brome cover with abundances of the most common bacterial phyla (*Acidobacteria*, *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, and *Verrucomicrobia*, Appendix Tables 5.7, 5.8).

High plant shoot and litter production is typically associated with competitive exclusion and reduced diversity in the plant community (Grace, 1999; Grime, 1973; Rout and Callaway, 2009). In this system, increased litter production by smooth brome was the dominant driver of reduced plant richness. Litter production likely suppressed native plant species through mechanisms such as shading or alteration of the physical environment (Facelli and Pickett, 1991; Lamb, 2008; Loydi et al., 2013; Xiong and Nilsson, 1999). Native grasses, such as *Hesperostipa comata, Hesperostipa curtiseta,* and *Elymus lanceolatus* ssp*. lanceolatus*,

along with native forbs, such as *Agoseris glauca, Androsace sepentrionalis, Artemisia frigida,* and *Viola adunca* appeared to be strongly impacted by this altered litter environment as they were strongly negatively associated with smooth brome cover (Tables 5.5, 5.6). The high quantity of shoot and litter production in smooth brome-dominated plots relative to the native plant community (Chapter Two, Fink and Wilson, 2011) may have supported a more diverse bacterial community through increased organic substrate (i.e. soil organic carbon and nitrogen), and resulting increase in niche availability (de Vries et al., 2012; Fierer et al., 2007; Hooper et al., 2000; Ramirez et al., 2010; Zhou et al., 2002). The SEM models did not support this hypothesis, despite the strong influence of smooth brome on soil organic carbon and nitrogen. However, these models did confirm the presence of an alternative, unknown mechanism linking plant community composition to bacterial community structure.

There are at least two possible explanations for these pathways, the first being changes to root exudate profiles. Although total soil organic carbon can be ruled out as a mechanism driving bacterial community structure, changes in the abundance or chemical profile of labile carbon released as root exudates from the plant community throughout the soil profile cannot be ruled out. Release of a unique combination or quantity of root exudates by smooth brome is supported indirectly by the fact that the relationship between smooth brome and bacterial community structure did not differ between horizons, despite significant differences in edaphic factors. Changes in root exudates may have been masked in this study, as the different pools of organic carbon were not distinguished. A second hypothesis for

these pathways suggests alterations to soil food web dynamics. A previous study of *Bromus tectorum* found that the soil food web was changed by invasion (Belnap and Phillips, 2001), so it is possible that smooth brome may be indirectly influencing the bacterial community through changes to soil food web structure. As bacterial biomass is typically controlled through top-down processes (Wardle, 2002), changes in the abundance or composition of bacterial-feeding nematodes, for example, may have important consequences for bacterial community structure. Evidence is currently insufficient to distinguish between these hypotheses, but these suggested mechanisms provide interesting, testable questions for future studies.

Shifts in plant community composition following smooth brome invasion were associated with altered abundances of multiple bacterial groups, including those important for nitrogen cycling. Both ammonia-oxidizing bacteria in the *Nitrosomonadales* (*Proteobacteria*) order, and nitrifying bacteria in the *Nitrospirales* (*Nitrospirae*) order were negatively associated with increasing smooth brome cover. In line with these responses, a sparse number of studies (but see Chapter 2, Hawkes et al., 2005) have also found altered populations of N-cycling bacteria with invasion. In Chapter Two, *amoA*, an indicator of ammonia-oxidizing bacteria (AOB) and archaea (AOA) population sizes, increased with smooth brome cover, which is opposite to the responses in this study. This discrepancy may be due to two factors. First, *Nitrospirales* also includes non-nitrifying bacteria, such as the family *Thermodesulfovibrionaceae*. Secondly, AOA and AOB species contain differing copy numbers of the gene target *amoA* (Norton et al., 2002), and changes in the abundance of *amoA* may be a function of changes in the diversity, as well as in

population sizes. *Rhizobiales* (*Proteobacteria*), an order containing N-fixing bacteria typically associated with legumes (amongst other non-N fixing families), was positively associated with smooth brome cover in the B horizon. As legumes root deeper in the soil profile (Craine et al., 2003), altered *Rhizobiales* abundance may be associated with changing legume root abundance or nodulation rate. Although aboveground legume cover was not, or only very weakly associated with smooth brome cover (Appendix Tables 5.5, 5.6), legumes did co-occur with brome, suggesting altered legume root biomass or nodulation unrelated to aboveground cover. As increasing soil N can inhibit nitrogen-fixing bacteria activity (Ledgard and Steele, 1992), greater *Rhizobiales* abundance may indicate increased competition for nitrogen.

As the composition and structure of the soil bacterial community can be a predictor of ecosystem function (Bell et al., 2005; Fierer et al., 2007; Strickland et al., 2009; Wittebolle et al., 2009; Zak et al., 2003), these results imply that brome invasion has important consequences for soil processes. Previous work (see Chapter Two) has shown that mineralization rates are higher in smooth brome invaded soils relative to native grassland. As the affinity for soil resources differ between bacterial species (e.g. Eilers et al., 2010; Fierer et al., 2007), a more even bacterial community may have promoted higher mineralization rates through increased partitioning and utilization of soil resources. Mineralization rates may have also been associated with the altered abundance of the multiple taxonomic groups that changed with smooth brome invasion, such as the candidate phylum CCM11b, or the group of unclassified Bacteria. However, the functional attributes of these groups, along with the vast majority of bacteria present in the soil are unknown. It is reasonable to presume

that one or more ecosystem functions may be modified by changes in their populations, but until research further elucidates the ecological roles of these bacteria, taxonomy cannot be linked to function.

In summary, smooth brome strongly influenced bacterial community structure and composition. These results suggest that not only is the bacterial community responsive to shifts in plant community composition caused by invasion, but that these changes may have important consequences for ecosystem processes such as nitrogen cycling. Examination of the labile carbon pools and other biological components of the soil may further elucidate the mechanisms linking plant community composition and soil bacterial structure. Examination of the temporal variability in these relationships may reveal the long-term legacy of smooth brome invasion on grassland ecosystem function.

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4 General Conclusions

The research presented in this thesis demonstrates strong links between the plant and soil communities in this Fescue Grassland. Chapter Two demonstrated that soil nitrogen cycling rates were influenced by changes in aboveground plant community composition and productivity. Specifically, the higher mineralization rates in smooth brome-invaded sites were linked to increased plant inputs to the soil (e.g. litter, roots), which in turn likely stimulated activity in the soil microbial community. Chapter Three examined the effects of smooth brome invasion on the structure of the soil bacterial community. Bacterial communities were more even in smooth brome-invaded areas relative to native grassland, as rare bacterial species became more abundant in smooth brome invaded soils. A more even bacterial community may have supported the higher mineralization rates seen in Chapter Two via increased resource utilization and niche partitioning. These results highlight the cascade of changes in the soil ecosystem induced by smooth brome invasion.

4.1 The effects of smooth brome on the aboveground plant community

Smooth brome strongly influenced the structure of the plant community. Similar to previous studies plant species richness and evenness were lower in smooth brome-dominated communities relative to native grassland (Fink and Wilson, 2011; Otfinowski et al., 2007; Romo et al., 1990). The SEM in Chapter Three suggested that reduced plant diversity was likely an indirect effect of smooth brome

driven by increased litter production. Increasing litter may have suppressed native plant species through mechanisms such as shading or alteration of the physical environment (Facelli and Pickett, 1991; Lamb, 2008; Loydi et al., 2013; Xiong and Nilsson, 1999). As a rhizomatous, clonal plant, smooth brome may be less affected by its own litter production as established clones can support the growth of newly developing ramets (Otfinowski and Kenkel, 2008). This integration of ramets may be an important invasion mechanism (Otfinowski and Kenkel, 2008), and demonstrates that not only is native plant growth suppressed by litter production, but that the clonal growth form of smooth brome may also provide a competitive advantage.

The greater litter production was caused by higher shoot and root production in smooth brome invaded areas, a common relationship in many invaded plant communities (Ehrenfeld, 2003, 2010; Fink and Wilson, 2011). Although the dominant mechanism by which smooth brome influenced plant diversity appears to be litter, greater shoot production suggests the potential for increased light competition. As shoot competition is size-asymmetric, with larger plants capturing disproportionately more light (Lamb et al., 2009), greater production of smooth brome shoot biomass may reduce plant diversity through increased aboveground competition. Increased root biomass in invaded areas also suggests a greater capacity for resource uptake by smooth brome. Although the role of root competition in structuring plant communities may not be directly important (Lamb and Cahill, 2008; Lamb et al., 2009), competition belowground can influence the outcome of shoot competition (Lamb et al., 2009). Finally, as plant roots are in direct

contact with the soil, relationships between plant and soil bacterial communities are likely to be mediated through changes in the root community.

4.2 The effects of smooth brome on the soil bacterial community

Shifts in the aboveground plant community and changes to productivity with smooth brome invasion resulted in significant changes belowground. Soil bacterial communities were more even and had higher species richness in smooth brome invaded areas relative to native fescue grassland. Increased species richness, rather than representing greater species numbers per se, is due likely to increased abundance of rare species (resulting in a greater probability of detection). As well as altered bacterial community structure, several taxonomic groups, including bacteria important for nitrogen cycling processes were influenced by changes in the aboveground plant community.

High plant community productivity may have supported greater bacterial diversity through increased soil organic matter inputs and the resulting increase in niche availability (Hooper et al., 2000). Although organic carbon, total nitrogen and root biomass were not predictors of bacterial community structure, structural equation modeling supported an alternative, unknown mechanism linking plant and bacterial communities. In Chapter Three, two potential explanations for this unknown mechanism were hypothesized. Bacterial community composition can be influenced by the abundance or chemical profile of labile carbon released as root exudates from the plant community (Bais et al., 2006; Eilers et al., 2010; Fierer et al., 2007; Lynch and Whipps, 1990). Although total soil organic carbon may be excluded as a mechanism linking plant and soil bacterial communities, this coarse measure

may have masked changes in the abundance or chemical profile of labile carbon. The second alternative hypothesis proposes an indirect influence of smooth brome on bacterial community structure through alterations of soil food web structure. As bacterial biomass is typically controlled through top-down processes (Wardle, 2002), changes in the abundance or composition of bacterial-feeding nematodes, for example, may have important consequences for bacterial community structure. Although other soil microbial components were not studied, a previous study of *Bromus tectorum* found that food web structure was changed by invasion (Belnap and Phillips, 2001). Evidence to distinguish between these hypotheses is insufficient, but these suggested mechanisms provide testable questions for future studies.

4.3 Effects of a changing plant and soil community on soil nitrogen cycling

Soil nitrogen cycling is an important process as productivity in these grasslands is typically limited by N availability (Lamb, 2008; Vitousek and Howarth, 1991). As plant community composition can influence soil nitrogen cycling rates (Craine et al., 2002; Knops et al., 2002; Scherer-Lorenzen, 2008; Wardle, 2002; Wedin and Tilman, 1990), changes in the plant community with smooth brome invasion may cause important consequences for soil nitrogen cycling. This hypothesis is supported by the responses reported in Chapter Two, which found that smooth brome-invaded areas had higher gross nitrogen mineralization rates relative to native fescue grassland. Greater mineralization rates are likely the product of a microbial community stimulated by the increased shoot, litter and root biomass in invaded areas. As the soil bacterial community is primarily responsible

for the mineralization of nitrogen, changes in the structure or abundance the bacterial community may be responsible for altered cycling rates.

In this thesis, multiple measures indicated that smooth brome could influence soil nitrogen cycling via changes in soil bacterial communities. The third chapter demonstrated that the bacterial community was more even in invaded soils due to an increase in the abundance of rare species. As the affinity for soil resources differ between bacterial species (e.g. Eilers et al., 2010; Fierer et al., 2007), a more diverse bacterial community may have promoted higher mineralization rates through increased partitioning and utilization of soil resources. Smooth brome invaded soils were also associated with increased abundance of both ammonia-oxidizing bacteria (AOB) and archaea (AOA) and nitrifying bacteria. In Chapter Two, *amoA*, an indicator of AOA and AOB population sizes, were found to increase with smooth brome cover, while in Chapter Three, the *Nitrosomonadales* and *Nitrospirales* orders were negatively associated with increasing smooth brome cover. This discrepancy between measurements may be due to two factors. First, *Nitrospirales* includes non-nitrifying bacteria, such as the family *Thermodesulfovibrionaceae*. Secondly, bacteria species contain differing copy numbers of the gene target *amoA* (Norton et al., 2002), and changes in the abundance of *amoA* may be a function of changes in the diversity, as well as changes in population sizes. This may explain why no changes in nitrification rates were observed, but despite this, these responses highlight the direct interaction of the plant community and bacteria with demonstrated roles in soil nitrogen cycling.

Altered plant community composition was also associated with several bacterial phyla and orders, such as *Chlamydiae* and the candidate phylum CCM11b. The functional role of these bacteria in the ecosystem are unknown (Fierer et al., 2007; Torsvik et al., 2002), but it is reasonable to presume that one or more ecosystem functions may be modified by changes in their populations. Future examination of the ecology of these bacteria may allow further insight into the ecological impact of the interactions between plant and soil communities.

4.4 Future research

This thesis demonstrated important changes occurring belowground with smooth brome invasion, highlighting different avenues of future research. In Chapter Two, how N cycling rates differed at the time of peak biomass production were examined, but it may also be informative to examine how N cycling rates differ across the growing season. In particular, examination of N cycling rates in spring may be useful as smooth brome is known to have rapid growth during this time period (Otfinowski et al., 2007). Studies of N cycling rates at the edge of invasion patches, or in newly colonized areas would provide important information of how N cycling rates compare before and after invasion.

Soil bacterial communities were altered with smooth brome invasion and in particular, rare species became more abundant. As the ecological role of many bacteria species are currently unknown, future research would benefit from a greater understanding of the roles of these species and the degree of functional redundancy amongst different bacteria species. This knowledge would not only give insight to why different bacterial species respond to a changing soil environment,

but also provide information about the ecological significance of these changes. Root exudates were also mentioned as a potential link between plant and soil bacterial communities. Examinations of labile carbon pools, especially as can be linked to root exudate profiles, may explain the unknown pathway linking the plant and soil bacterial communities. Finally, although the bacterial community typically dominates the microbial biomass (Fierer and Lennon, 2011), exploration of the response of soil ecosystem components such as archaea and soil nematodes to smooth brome invasion may identify further impacts of smooth brome on soil microbial community structure. This research will add to the growing evidence of the strong influence and potential legacy effects of smooth brome on native grasslands.

4.5 References

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5 Appendix

Table 5.1 Unstandardized and standardized path coefficients, standard error of unstandardized coefficients and significance tests for the bacterial richness multi-group structural equation model. Paths run from the variables below bolded text to the variable in bold. One ~ symbol indicates a directed relationship and two ~ indicates an undirected covariance. In cases where model fit required different path coefficients between horizons, path coefficients for each horizon are indicated by a superscript A or B.

	Unstandardized estimate	Standard error	Z value	P value	Standardized estimate
Plant Richness~					
A Horizon	0.144	0.071	2.04	0.042	0.169
Brome shoot biomass	-23.81	16.41	-1.45	0.147	-0.143
Litter biomass	-81.09	21.86	-3.71	0.000	-0.366
Litter C:N Ratio~					
Brome shoot biomass	25.66	25.38	1.01	0.312	0.097
Plant richness	-0.306	0.153	-2.00	0.045	-0.193
Litter biomass~					
Brome shoot biomass	0.420	0.058	7.23	0.000	0.559
log(Root Biomass)~					
Brome shoot biomass	10.14	2.30	4.42	0.000	0.426
Plant richness	0.023	0.014	1.68	0.092	0.163
Soil Organic Carbon~					

	Unstandardized estimate	Standard error	Z value	P value	Standardized estimate
Litter C:N ratio[A]	0.037	0.021	1.76	0.078	0.116
Litter C:N ratio[B]	0.022	0.014	1.53	0.126	0.158
Litter biomass	19.67	4.95	3.98	0.000	0.174
log(Root biomass)	0.302	0.141	2.14	0.033	0.085
Total Soil Nitrogen~					
Litter C:N ratio	0.003	0.002	1.65	0.099	0.094
Litter biomass	1.74	0.558	3.11	0.002	0.171
log(Root biomass)	0.035	0.016	2.15	0.032	0.108
Microbe Richness~					
Soil organic carbon	-0.003	0.003	-1.08	0.282	-0.423
Total soil nitrogen	0.038	0.029	1.34	0.179	0.505
log(Root biomass)	0.002	0.002	0.851	0.395	0.073
Soil Organic Carbon~~					
Total soil Nitrogen[A]	0.265	0.05	5.26	0.000	0.956
Total soil Nitrogen[B]	0.048	0.009	5.14	0.000	0.946

Table 5.2 Unstandardized and standardized path coefficients, standard error of unstandardized coefficients and significance tests for the bacterial community evenness multi-group structural equation model. Paths run from the variables below bolded text to the variable in bold. One ~ symbol indicates a directed relationship and two ~ indicates an undirected covariance. In cases where model fit required different path coefficients between horizons, path coefficients are shown for both, and are indicated by a superscript A or B.

	Unstandardized estimate	**Standard error**	**Z-value**	**P value**	**Standardized estimate**
Plant Richness~					
A Horizon	0.144	0.071	2.04	0.042	0.169
Litter biomass	-81.10	21.86	-3.71	0.000	-0.366
Litter C:N ratio~					
Brome shoot biomass	25.66	25.38	1.01	0.312	0.097
Plant richness	-0.306	0.153	-2.00	0.045	-0.193
Litter biomass					
Brome shoot biomass	0.420	0.058	7.23	0.000	0.559
log(Root biomass)~					
Brome shoot biomass	10.13	2.29	4.43	0.000	0.425
Plant richness	0.023	0.014	1.68	0.092	0.163
Soil Organic Carbon~					
Litter C:N ratio^{A}	0.037	0.021	1.76	0.078	0.116
Litter C:N ratio^{B}	0.022	0.014	1.53	0.126	0.157
Litter biomass	19.67	4.96	3.96	0.000	0.174
log(Root biomass)	0.302	0.142	2.13	0.033	0.085
Total Soil Nitrogen~					

	Unstandardized estimate	Standard error	Z-value	P value	Standardized estimate
Litter C:N ratio	0.003	0.002	1.65	0.099	0.094
Litter biomass	1.74	0.559	3.11	0.002	0.171
log(Root biomass)	0.035	0.016	2.14	0.032	0.108
Bacterial Evenness~					
Soil organic carbon	-0.008	0.011	-0.736	0.462	-0.299
Total soil nitrogen	0.121	0.118	1.028	0.304	0.404
log(Root biomass)	0.003	0.009	0.358	0.720	0.033
Soil Organic Carbon~~					
Total soil nitrogen[A]	0.265	0.050	5.26	0.000	0.956
Total soil nitrogen[B]	0.048	0.009	5.14	0.000	0.946

Table 5.3 Unstandardized and standardized path coefficients, standard error of unstandardized coefficients and significance tests for the bacterial community richness multi-group structural equation model with direct paths from smooth brome biomass and plant richness added in. Paths run from the variables below bolded text to the variable in bold. One ~ symbol indicates a directed relationship and two ~ indicates an undirected covariance. In cases where model fit required different path coefficients between horizons, path coefficients are shown for both, and are indicated by a superscript A or B.

	Unstandardized estimate	**Standard error**	**Z-value**	**P value**	**Standardized estimate**
Plant Richness~					
A Horizon	0.144	0.071	2.04	0.042	0.169
Brome shoot biomass	-23.81	16.41	-1.45	0.147	-0.143
Litter biomass	-81.09	21.86	-3.71	0.000	-0.366
Litter C:N Ratio~					
Brome shoot biomass	25.66	25.38	1.01	0.312	0.097
Plant richness	-0.306	0.153	-2.00	0.045	-0.193
Litter biomass					
Brome shoot biomass	0.420	0.058	7.23	0.000	0.559
log(Root biomass)~					
Brome shoot biomass	10.14	2.30	4.42	0.000	0.426
Plant richness	0.023	0.014	1.68	0.092	0.163
Soil Organic Carbon~					
Litter C:N ratio^{A}	0.037	0.021	1.76	0.078	0.116
Litter C:N ratio^{B}	0.022	0.014	1.53	0.126	0.158
Litter biomass	19.67	4.95	3.98	0.000	0.174
log(Root biomass)	0.302	0.141	2.14	0.033	0.085

	Unstandardized estimate	Standard error	Z-value	P value	Standardized estimate
Total Soil Nitrogen~					
Litter C:N ratio	0.003	0.002	1.65	0.099	0.094
Litter biomass	1.74	0.558	3.11	0.002	0.171
log(Root biomass)	0.035	0.016	2.15	0.032	0.108
Microbe Richness~					
Brome shoot biomass	0.115	0.057	2.03	0.043	0.207
Soil organic carbon	-0.003	0.002	-1.40	0.161	-0.533
Total soil nitrogen	0.039	0.027	1.46	0.144	0.535
Plant richness	-0.001	0.000	-1.92	0.055	-0.184
log(Root biomass)	0.000	0.002	0.231	0.817	0.021
Soil Organic Carbon~~					
Total Soil Nitrogen[A]	0.265	0.050	5.26	0.000	0.956
Total Soil Nitrogen[A]	0.048	0.009	5.14	0.000	0.946

Table 5.4 Unstandardized and standardized path coefficients, standard error of unstandardized coefficients and significance tests for the bacterial community evenness multi-group structural equation model with direct paths from smooth brome shoot biomass and plant species richness to bacterial richness added in. Paths run from the variables below bolded text to the variable in bold. One ~ symbol indicates a directed relationship and two ~ indicates an undirected covariance. In cases where model fit required different path coefficients between horizons, path coefficients are shown for both, and are indicated by a superscript A or B.

	Unstandardized estimate	Standard error	Z-value	P value	Standardized estimate
Plant Richness~					
A Horizon	0.144	0.071	2.04	0.042	0.169
Brome shoot biomass	-23.81	16.41	-1.45	0.147	-0.143
Litter biomass	-81.09	21.86	-3.71	0.000	-0.366
Litter C:N Ratio~					
Brome shoot biomass	25.66	25.38	1.01	0.312	0.097
Plant richness	-0.306	0.153	-2.00	0.045	-0.193
Litter biomass					
Brome shoot biomass	0.420	0.058	7.23	0.000	0.559
log(Root biomass)~					
Brome shoot biomass	10.14	2.30	4.42	0.000	0.426
Plant richness	0.023	0.014	1.68	0.092	0.163
Soil Organic Carbon~					
Litter C:N ratio^{A}	0.037	0.021	1.76	0.078	0.116
Litter C:N ratio^{B}	0.022	0.014	1.53	0.126	0.158

	Unstandardized estimate	Standard error	Z-value	P value	Standardized estimate
Litter biomass	19.67	4.95	3.98	0.000	0.174
log(Root biomass)	0.302	0.141	2.14	0.033	0.085
Total Soil Nitrogen~					
Litter C:N ratio	0.003	0.002	1.65	0.099	0.094
Litter biomass	1.74	0.558	3.11	0.002	0.171
log(Root biomass)	0.035	0.016	2.15	0.032	0.108
Microbe Evenness~					
Brome shoot biomass	0.570	0.238	2.39	0.017	0.255
Soil organic carbon	-0.012	0.010	-1.19	0.234	-0.458
Total soil nitrogen	0.139	0.109	1.280	0.201	0.478
Plant richness	-0.003	0.001	-1.95	0.051	-0.196
log(Root biomass)	-0.003	0.009	-0.325	0.746	-0.031
Total Soil Nitrogen[A]	0.265	0.050	5.26	0.000	0.956
Soil Organic Carbon~~					
Total Soil Nitrogen[A]	0.048	0.009	5.14	0.000	0.946

Table 5.5 Correlations of A horizon NMS axes with plant species cover. Plant names are according to the International Taxonomic Information System (ITIS).

	Axis 1			Axis 2		
Species	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Achillea millefolium	-0.054	0.003	-0.096	0.190	0.036	0.083
Agoseris glauca	-0.438	0.192	-0.377	0.056	0.003	-0.058
Agrostis scabra	-0.102	0.010	-0.107	-0.343	0.118	-0.237
Androsace sepentrionalis	-0.359	0.129	-0.234	-0.251	0.063	-0.153
Anemone candensis	-0.037	0.001	-0.094	-0.004	0.000	0.054
Anemone patens	-0.169	0.029	-0.164	-0.317	0.101	-0.007
Antennaria parvifolia	-0.342	0.117	-0.228	0.012	0.000	-0.009
Arabis hirsuta	-0.118	0.014	-0.153	0.226	0.051	0.146
Artemisia frigida	-0.432	0.187	-0.374	-0.048	0.002	-0.103
Artemisia ludoviciana	-0.058	0.003	-0.040	-0.290	0.084	-0.135
Astragalus agrestis	0.026	0.001	-0.040	0.185	0.034	0.133
Astragalus flexuosus	-0.277	0.077	-0.209	-0.322	0.104	-0.281
Astragalus laxmannii var. robustior	-0.190	0.036	-0.134	0.174	0.030	0.153
Avenula hookeri	-0.350	0.123	-0.267	-0.407	0.165	-0.220
Boechera divaricarpa	-0.281	0.079	-0.179	-0.099	0.010	-0.134
Bouteloua gracilis	-0.198	0.039	-0.140	0.116	0.013	0.121
Bromus anomalus	0.077	0.006	-0.048	-0.095	0.009	-0.065
Bromus inermis	0.886	0.786	0.839	-0.108	0.012	-0.068
Campanula rotundifolia	0.041	0.002	-0.003	0.146	0.021	0.164
Carex duriscula	-0.048	0.002	0.021	0.017	0.000	-0.010
Carex filifolia	-0.362	0.131	-0.161	0.037	0.001	-0.105
Carex obtusata	0.025	0.001	-0.041	0.097	0.009	0.103

	Axis 1			Axis 2		
Species	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Carex pensylvanica	-0.196	0.038	-0.099	-0.401	0.160	-0.338
Carex species	-0.141	0.020	-0.107	-0.394	0.156	-0.178
Cerastium arvense	0.150	0.022	0.097	-0.053	0.003	-0.010
Cirsium flodmanii	0.075	0.006	0.038	-0.097	0.009	0.026
Descurainia sophia	-0.046	0.002	-0.081	0.190	0.036	0.166
Elymus lanceolatus ssp. lanceolatus	-0.576	0.332	-0.468	0.034	0.001	-0.187
Elymus trachycaulus ssp. subsecundus	-0.159	0.025	-0.158	-0.192	0.037	0.020
Erigeron glabellus	-0.033	0.001	-0.068	0.132	0.017	0.127
Erysimum inconspicuum	-0.039	0.001	-0.088	0.199	0.039	0.195
Festuca altaica ssp. hallii	-0.122	0.015	-0.188	0.649	0.421	0.718
Galium boreale	0.024	0.001	0.099	-0.061	0.004	0.049
Gentianella amarella ssp. acuta	0.097	0.009	0.055	-0.008	0.000	0.029
Geum trifolium	0.097	0.009	0.055	-0.008	0.000	0.029
Hesperostipa comata	-0.544	0.296	-0.452	-0.190	0.036	-0.283
Hesperostipa curtiseta	-0.443	0.196	-0.247	0.185	0.034	0.114
Juncus balticus	-0.118	0.014	-0.027	-0.295	0.087	-0.096
Koeleria macrantha	-0.264	0.070	-0.186	-0.129	0.017	-0.147
Linum lewisii	0.049	0.002	-0.029	-0.149	0.022	-0.159
Melilotus officinalis	-0.201	0.040	-0.147	-0.227	0.051	-0.173
Muhlenbergia richardsonis	-0.156	0.024	-0.127	-0.324	0.105	-0.179
Mulgedium oblongifolium	0.138	0.019	0.186	-0.031	0.001	-0.062
Nassella viridula	-0.107	0.011	0.026	-0.404	0.163	-0.158
Pascopyrum smithii	-0.382	0.146	-0.415	-0.372	0.139	-0.048
Pediomelum argophyllum	0.084	0.007	0.023	-0.065	0.004	-0.114
Penstemon procerus	0.104	0.011	0.114	-0.075	0.006	0.019
Poa palustris	-0.140	0.020	-0.116	0.010	0.000	-0.014
Poa pratensis	0.136	0.018	0.098	0.112	0.012	0.074

	Axis 1			Axis 2		
Species	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Rosa arkansana	0.168	0.028	0.201	0.156	0.024	0.113
Rosa woodsii	0.115	0.013	0.061	0.011	0.000	-0.061
Salix wolfii	0.052	0.003	-0.010	0.099	0.010	0.114
Solidago missouriensis	-0.131	0.017	-0.066	-0.092	0.008	-0.063
Sonchus arvensis	0.067	0.005	0.057	0.120	0.014	0.206
Sphaeralcea coccinea	-0.311	0.096	-0.186	0.088	0.008	0.101
Stellaria longifolia	-0.122	0.015	-0.121	-0.425	0.181	-0.174
Symphoriocarpos occidentalis	0.290	0.084	0.274	-0.016	0.000	-0.004
Symphotrichum ericoides	0.108	0.012	0.239	0.059	0.003	0.056
Symphotrichum laeve	0.107	0.012	0.027	0.241	0.058	0.252
Taraxacum officinale	-0.029	0.001	-0.093	0.169	0.029	0.170
Thalictrum venulosum	0.084	0.007	0.023	-0.065	0.004	-0.114
Thermopsis rhombifolia	0.033	0.001	0.195	0.252	0.063	-0.034
Tragopogon dubius	-0.102	0.010	-0.127	0.184	0.034	0.141
Vicia americana var. minor	-0.250	0.062	-0.258	0.042	0.002	-0.037
Viola adunca	-0.304	0.092	-0.259	0.229	0.053	0.215

Table 5.6 Correlations of B horizon NMS axes with plant species cover. Naming system is according to the International Taxonomic Information System (ITIS).

	Axis 1			Axis 2		
Species	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Achillea millefolium	-0.085	0.007	-0.123	0.193	0.037	0.089
Agoseris glauca	-0.447	0.200	-0.373	-0.054	0.003	-0.115
Agrostis scabra	-0.066	0.004	-0.086	-0.369	0.136	-0.182
Androsace sepentrionalis	-0.334	0.111	-0.232	-0.351	0.123	-0.235
Anemone canadensis	-0.040	0.002	-0.088	-0.005	0.000	0.031
Anemone patens	-0.177	0.031	-0.164	-0.321	0.103	-0.007
Antennaria parvifolia	-0.356	0.126	-0.240	-0.097	0.009	-0.049
Arabis hirsuta	-0.136	0.018	-0.157	0.165	0.027	0.120
Artemesia frigida	-0.415	0.172	-0.369	-0.186	0.035	-0.179
Artemesia ludoviciana	0.055	0.003	-0.005	-0.114	0.013	-0.089
Astragalus agrestis	0.002	0.000	-0.054	0.224	0.050	0.172
Astragalus flexuosus	-0.218	0.047	-0.172	-0.392	0.154	-0.285
Astragalus laxmannii var. robustior	-0.229	0.053	-0.148	0.092	0.008	0.113
Avenula hookeri	-0.261	0.068	-0.223	-0.488	0.238	-0.220
Boechera divaricarpa	-0.265	0.070	-0.182	-0.177	0.031	-0.148
Bouteloua gracilis	-0.233	0.054	-0.155	0.041	0.002	0.079
Bromus anomalus	0.091	0.008	-0.017	-0.067	0.005	-0.027
Bromus inermis	0.904	0.817	0.828	0.122	0.015	0.007
Campanula rotundifolia	-0.009	0.000	-0.041	0.129	0.017	0.116
Carex duriscula	-0.131	0.017	-0.049	-0.027	0.001	-0.108
Carex filifolia	-0.351	0.123	-0.164	-0.097	0.009	-0.183
Carex obtusata	0.032	0.001	-0.005	0.176	0.031	0.178

	Axis 1			Axis 2		
Species	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Carex pensylvanica	-0.099	0.010	-0.064	-0.447	0.200	-0.251
Carex species	-0.123	0.015	-0.085	-0.381	0.145	-0.229
Cerastium arvense	0.163	0.027	0.127	0.069	0.005	0.099
Cirsium flodmanii	0.117	0.014	0.051	-0.050	0.003	0.128
Descurainia sophia	-0.068	0.005	-0.093	0.171	0.029	0.161
Elymus lanceolatus ssp. lanceolatus	-0.572	0.328	-0.455	-0.166	0.028	-0.364
Elymus trachycaulus ssp. subsecundus	-0.167	0.028	-0.142	-0.187	0.035	0.056
Erigeron glabellus	-0.024	0.001	-0.052	0.175	0.031	0.168
Erysimum inconspicuum	-0.055	0.003	-0.088	0.194	0.038	0.206
Festuca altaica ssp. hallii	-0.231	0.053	-0.254	0.555	0.308	0.625
Galium boreale	0.051	0.003	0.115	-0.036	0.001	0.129
Gentianella amarella ssp. acuta	0.102	0.010	0.058	0.017	0.000	0.024
Geum trifolium	0.102	0.010	0.058	0.017	0.000	0.024
Hesperostipa comata	-0.514	0.264	-0.454	-0.359	0.129	-0.369
Hesperostipa curtiseta	-0.474	0.225	-0.287	0.037	0.001	0.040
Juncus balticus	-0.043	0.002	-0.014	-0.335	0.112	-0.159
Koeleria macrantha	-0.236	0.056	-0.180	-0.221	0.049	-0.236
Linum lewisii	0.078	0.006	-0.006	-0.144	0.021	-0.172
Melilotus officinalis	-0.165	0.027	-0.127	-0.272	0.074	-0.175
Muhlenbergia richardsonis	-0.066	0.004	-0.086	-0.369	0.136	-0.182
Mulgedium oblongifolium	0.152	0.023	0.189	0.012	0.000	0.003
Nassella viridula	-0.109	0.012	0.003	-0.390	0.152	-0.148
Pascopyrum smithii	-0.367	0.135	-0.408	-0.459	0.211	-0.141
Pediomelum argophyllum	0.092	0.009	0.038	-0.045	0.002	-0.113
Penstemon procerus	0.116	0.013	0.112	0.112	0.013	0.180
Poa palustris	-0.138	0.019	-0.118	-0.039	0.001	-0.025
Poa pratensis	0.128	0.016	0.080	0.133	0.018	0.071

	Axis 1			Axis 2		
Species	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Rosa arkansana	0.124	0.015	0.202	0.238	0.057	0.212
Rosa woodsii	0.101	0.010	0.051	0.041	0.002	0.027
Salix wolfii	0.000	0.000	0.000	0.000	0.000	0.000
Solidago missouriensis	-0.096	0.009	-0.046	-0.134	0.018	-0.126
Sonchus arvensis	0.059	0.003	0.063	0.295	0.087	0.410
Sphaeralcea coccinea	-0.305	0.093	-0.189	-0.022	0.000	-0.079
Stellaria longifolia	-0.064	0.004	-0.083	-0.425	0.181	-0.164
Symphoriocarpos occidentalis	0.285	0.081	0.320	0.142	0.020	0.169
Symphotrichum ericoides	0.111	0.012	0.236	0.076	0.006	0.086
Symphotrichum laeve	0.067	0.005	0.003	0.253	0.064	0.242
Taraxacum officinale	-0.021	0.000	-0.083	0.132	0.017	0.142
Thalictrum venulosum	0.092	0.009	0.038	-0.045	0.002	-0.113
Thermopsis rhombifolia	-0.011	0.000	0.255	0.309	0.096	0.128
Tragopogon dubius	-0.146	0.021	-0.183	0.121	0.015	0.141
Vicia americana var. minor	-0.261	0.068	-0.308	-0.032	0.001	-0.075
Viola adunca	-0.325	0.106	-0.274	0.124	0.015	0.102

Table 5.7 Correlations between the abundance of bacterial phyla with NMS axes for A horizon.

	Axis 1			Axis 2		
Phylum	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Acidobacteria	0.059	0.003	0.037	0.053	0.003	0.012
Actinobacteria	-0.026	0.001	-0.054	-0.024	0.001	-0.017
AD3	0.072	0.005	-0.003	0.065	0.004	0.088
Aquificae	0.122	0.015	0.028	0.079	0.006	0.128
Armatimonadetes	-0.168	0.028	-0.121	0.007	0.000	-0.021
Bacteroidetes	0.117	0.014	0.134	0.078	0.006	0.033
BRC1	-0.126	0.016	-0.075	-0.021	0.000	-0.079
CCM11b	-0.503	0.253	-0.337	-0.237	0.056	-0.069
Chlamydiae	-0.222	0.049	-0.190	-0.181	0.033	-0.098
Chlorobi	-0.057	0.003	-0.004	-0.003	0.000	-0.028
Chloroflexi	0.083	0.007	0.078	-0.038	0.001	-0.089
Crenarchaeota	-0.219	0.048	-0.152	-0.070	0.005	-0.103
Cyanobacteria	-0.326	0.107	-0.161	-0.081	0.007	0.038
Deferribacteres	0.041	0.002	0.010	0.172	0.030	0.136
Dictyoglomi	-0.290	0.084	-0.208	0.023	0.001	-0.038
Elusimicrobia	0.158	0.025	0.059	0.044	0.002	0.001
Euryarchaeota	-0.122	0.015	-0.134	-0.098	0.010	-0.066
Fibrobacteres	0.068	0.005	0.136	0.150	0.023	0.089
Firmicutes	-0.182	0.033	-0.252	-0.391	0.153	0.037
GAL15	-0.061	0.004	0.032	-0.125	0.016	-0.058
Gemmatimonadetes	-0.240	0.058	-0.153	0.049	0.002	-0.110
GN02	-0.010	0.000	-0.025	0.030	0.001	-0.029
GN12	0.167	0.028	0.220	-0.014	0.000	0.021
GOUTA4	0.111	0.012	0.101	0.008	0.000	0.062
Unclassified Bacteria	0.375	0.140	0.278	-0.026	0.001	0.035
Lentisphaerae	-0.066	0.004	0.040	-0.046	0.002	-0.022
Nitrospirae	-0.421	0.178	-0.257	0.068	0.005	0.094
NKB19	0.114	0.013	0.121	0.050	0.003	0.135
OP3	0.140	0.019	0.087	-0.144	0.021	-0.090
Planctomycetes	0.239	0.057	0.205	0.184	0.034	0.042
Proteobacteria	0.113	0.013	0.096	0.157	0.025	0.089
SC3	0.151	0.023	0.211	-0.110	0.012	-0.102
SC4	-0.081	0.007	-0.107	0.062	0.004	0.084
SM2F11	-0.040	0.002	-0.100	0.117	0.014	0.100
SPAM	0.236	0.056	0.181	-0.069	0.005	-0.070
Spirochaetes	-0.151	0.023	-0.143	0.091	0.008	0.084
SR1	0.120	0.015	0.134	-0.031	0.001	-0.068

	Axis 1			Axis 2		
Phylum	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Synergistetes	0.030	0.001	0.020	0.213	0.045	0.130
Tenericutes	0.138	0.019	0.103	-0.012	0.000	0.033
Thermi	0.029	0.001	0.042	-0.141	0.020	-0.139
Thermotogae	-0.142	0.020	0.013	-0.060	0.004	-0.056
TM6	0.106	0.011	-0.002	0.052	0.003	-0.030
TM7	0.054	0.003	0.086	0.050	0.003	0.025
Verrucomicrobia	-0.053	0.003	-0.045	0.212	0.045	0.240
WPS-2	-0.001	0.000	-0.053	0.118	0.014	0.146
WS3	0.097	0.009	0.158	-0.296	0.087	-0.189
WS6	0.058	0.003	0.028	0.094	0.009	0.126
ZB2	-0.377	0.142	-0.257	-0.056	0.003	-0.074

Table 5.8 Correlations of bacterial phyla abundance with axes from B horizon NMS ordination.

	Axis 1			Axis 2		
Phylum	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Acidobacteria	0.000	0.000	-0.019	0.113	0.013	0.075
Actinobacteria	-0.049	0.002	-0.008	-0.117	0.014	-0.183
AD3	0.127	0.016	0.057	0.044	0.002	0.146
Aquificae	0.177	0.031	0.191	-0.014	0.000	-0.088
Armatimonadetes	-0.221	0.049	-0.157	-0.019	0.000	-0.083
Bacteroidetes	-0.034	0.001	-0.014	0.165	0.027	0.209
BRC1	0.079	0.006	0.015	0.119	0.014	0.083
CCM11b	-0.199	0.040	-0.124	-0.347	0.121	-0.317
Chlamydiae	-0.318	0.101	-0.230	-0.341	0.116	-0.266
Chlorobi	-0.014	0.000	-0.027	0.032	0.001	0.053
Chloroflexi	-0.206	0.043	-0.100	-0.094	0.009	-0.146
Crenarchaeota	0.307	0.094	0.220	-0.082	0.007	-0.153
Cyanobacteria	-0.149	0.022	-0.151	0.228	0.052	0.253
Deferribacteres	0.030	0.001	0.003	-0.003	0.000	-0.060
Dictyoglomi	0.000	0.000	0.000	0.000	0.000	0.000
Elusimicrobia	-0.200	0.040	-0.155	0.064	0.004	0.047
Euryarchaeota	0.058	0.003	0.050	0.219	0.048	0.190
Fibrobacteres	-0.033	0.001	0.021	0.145	0.021	0.039
Firmicutes	-0.050	0.003	-0.072	-0.200	0.040	-0.078
GAL15	-0.209	0.044	-0.113	-0.305	0.093	-0.241
Gemmatimonadetes	-0.251	0.063	-0.160	-0.145	0.021	-0.059
GN02	0.000	0.000	0.027	0.085	0.007	0.076
GN12	-0.062	0.004	-0.061	0.151	0.023	0.104
GOUTA4	0.196	0.039	0.245	0.021	0.000	0.020
Unclassified Bacteria	0.075	0.006	0.096	0.163	0.026	0.124
Lentisphaerae	0.000	0.000	0.000	0.000	0.000	0.000
Nitrospirae	-0.159	0.025	-0.033	-0.280	0.079	-0.166
NKB19	0.222	0.049	0.236	0.053	0.003	0.091
OP3	-0.106	0.011	-0.155	-0.143	0.020	-0.100
Planctomycetes	-0.234	0.055	-0.106	0.050	0.002	-0.033
Proteobacteria	0.047	0.002	-0.001	0.179	0.032	0.147
SC3	-0.027	0.001	0.012	0.163	0.027	0.167
SC4	0.082	0.007	0.088	0.082	0.007	0.118
SM2F11	-0.017	0.000	-0.053	0.008	0.000	-0.043
SPAM	-0.132	0.018	-0.087	-0.154	0.024	-0.154
Spirochaetes	0.028	0.001	-0.006	0.033	0.001	0.026
SR1	0.000	0.000	0.000	0.000	0.000	0.000

	Axis 1			Axis 2		
Phylum	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Synergistetes	-0.246	0.060	-0.236	0.050	0.002	0.022
Tenericutes	0.024	0.001	-0.003	-0.018	0.000	-0.003
Thermi	0.085	0.007	0.059	0.057	0.003	0.085
Thermotogae	0.100	0.010	0.069	0.164	0.027	0.026
TM6	-0.066	0.004	0.051	-0.021	0.000	0.019
TM7	0.104	0.011	0.034	0.134	0.018	0.128
Verrucomicrobia	0.161	0.026	0.183	0.075	0.006	0.136
WPS-2	-0.238	0.057	-0.161	-0.130	0.017	-0.134
WS3	0.012	0.000	0.044	0.061	0.004	0.036
WS6	0.112	0.013	0.091	-0.041	0.002	-0.034
ZB2	0.045	0.002	-0.003	0.076	0.006	0.100

Table 5.9 Correlations between bacterial orders and NMS axes for the A horizon ordination.

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
0319-7L14	-0.136	0.019	-0.110	-0.164	0.027	-0.082
32-20	0.298	0.089	0.224	-0.061	0.004	-0.081
A31	0.210	0.044	0.120	0.138	0.019	0.095
A4b	0.417	0.174	0.298	0.087	0.008	0.093
Acholeplasmatales	0.000	0.000	0.000	0.000	0.000	0.000
Acidimicrobiales	0.186	0.035	0.104	0.140	0.020	0.069
Acidobacteriales	0.112	0.012	0.101	0.008	0.000	-0.060
Actinomycetales	0.089	0.008	0.023	0.084	0.007	0.015
AKYG885	-0.268	0.072	-0.167	0.054	0.003	-0.048
Anaerolineales	-0.123	0.015	0.100	-0.066	0.004	-0.116
Aquificales	0.122	0.015	0.028	0.079	0.006	0.128
Armatimonadales	-0.101	0.010	-0.117	0.106	0.011	0.163
B07_WMSP1	0.387	0.150	0.301	0.085	0.007	0.073
Bacillales	-0.184	0.034	-0.272	-0.389	0.151	0.040
Bacteroidales	-0.011	0.000	-0.054	0.068	0.005	-0.061
Bdellovibrionales	0.195	0.038	0.206	-0.030	0.001	0.033
Brachyspirales	-0.017	0.000	0.075	-0.091	0.008	-0.136
Burkholderiales	0.248	0.061	0.130	0.057	0.003	0.020
Caldilineales	-0.053	0.003	-0.051	-0.088	0.008	-0.176
Campylobacterales	0.099	0.010	0.102	-0.062	0.004	-0.074
Cardiobacteriales	0.000	0.000	0.000	0.000	0.000	0.000
Caulobacterales	0.014	0.000	-0.004	-0.011	0.000	-0.041
CFB-26	0.237	0.056	0.201	0.023	0.001	0.051
Chlamydiales	-0.222	0.049	-0.190	-0.181	0.033	-0.098

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Chlorobiales	0.180	0.032	0.209	-0.022	0.000	-0.009
Chloroflexales	0.399	0.159	0.327	-0.092	0.009	-0.111
Chlorophyta	-0.372	0.138	-0.294	-0.070	0.005	0.031
Chromatiales	0.365	0.133	0.248	0.034	0.001	0.058
Chroococcales	0.160	0.025	0.158	-0.025	0.001	0.000
Chthonomonadales	-0.006	0.000	0.005	0.030	0.001	-0.022
CL500-15	0.273	0.075	0.249	-0.130	0.017	-0.193
Clostridiales	0.109	0.012	0.081	-0.194	0.038	-0.157
Coriobacteriales	0.055	0.003	-0.016	0.176	0.031	0.057
CTD005-82B-02	0.162	0.026	0.212	0.125	0.016	0.147
CV106	0.242	0.058	0.192	-0.162	0.026	-0.221
Deferribacterales	0.041	0.002	0.010	0.172	0.030	0.136
Dehalococcoidales	0.000	0.000	0.000	0.000	0.000	0.000
Deinococcales	0.029	0.001	0.042	-0.141	0.020	-0.139
Desulfovibrionales	0.119	0.014	0.076	0.132	0.018	0.023
Desulfurellales	-0.319	0.102	-0.239	-0.173	0.030	-0.118
Desulfuromonadales	0.170	0.029	0.143	0.050	0.003	0.118
Dictyoglomales	-0.290	0.084	-0.208	0.023	0.001	-0.038
DS-18	-0.199	0.040	-0.172	0.070	0.005	-0.015
Elusimicrobiales	0.000	0.000	0.000	0.000	0.000	0.000
Enterobacteriales	0.376	0.141	0.330	0.048	0.002	0.013
Entotheonellales	-0.106	0.011	-0.091	0.069	0.005	0.048
envOPS12	-0.024	0.001	0.009	-0.244	0.060	-0.135
Euglenozoa	0.093	0.009	0.049	0.022	0.000	0.075
Euzebiales	-0.280	0.078	-0.136	-0.130	0.017	-0.188
Exiguobacterales	0.005	0.000	-0.029	0.167	0.028	0.147
FAC88	0.003	0.000	-0.049	0.034	0.001	-0.064

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Fibrobacterales	0.068	0.005	0.136	0.150	0.023	0.089
Flavobacteriales	-0.032	0.001	-0.112	0.177	0.031	0.151
GCA004	0.185	0.034	0.175	-0.013	0.000	0.011
Gemata	0.202	0.041	0.075	0.018	0.000	0.002
Gemmatimonadales	-0.264	0.070	-0.206	0.058	0.003	-0.058
H39	-0.051	0.003	-0.021	0.174	0.030	0.192
Halanaerobiales	0.088	0.008	0.036	-0.035	0.001	-0.081
Halobacteriales	-0.124	0.015	-0.159	-0.176	0.031	-0.129
Herpetosiphonales	0.018	0.000	0.003	0.094	0.009	0.131
HN1-15	0.138	0.019	0.073	-0.033	0.001	-0.151
Holophagales	0.108	0.012	0.081	0.002	0.000	0.055
Hydrogenophilales	-0.173	0.030	-0.123	-0.073	0.005	-0.061
koll13	0.048	0.002	0.145	0.048	0.002	-0.056
Lactobacillales	0.003	0.000	-0.056	0.157	0.025	0.151
LD1-PA13	-0.143	0.020	0.014	-0.259	0.067	-0.195
Legionellales	0.180	0.032	0.104	0.338	0.114	0.238
Leptospirales	-0.158	0.025	-0.174	0.122	0.015	0.111
MC47	-0.020	0.000	-0.070	-0.046	0.002	-0.020
Methanosarcinales	-0.012	0.000	-0.009	0.192	0.037	0.060
Methylacidiphilales	-0.062	0.004	-0.104	-0.004	0.000	-0.044
Methylococcales	-0.270	0.073	-0.251	-0.029	0.001	-0.145
Methylophilales	-0.019	0.000	-0.075	-0.033	0.001	-0.101
MIZ46	0.236	0.056	0.119	-0.010	0.000	-0.001
mle1-12	-0.026	0.001	-0.058	-0.049	0.002	-0.044
mle1-48	0.040	0.002	0.084	0.135	0.018	0.084
MVP-88	0.201	0.040	0.085	0.031	0.001	0.041
Mycoplasmatales	0.138	0.019	0.103	-0.012	0.000	0.033

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Myxococcales	-0.297	0.088	-0.109	0.188	0.035	0.129
Natranaerobiales	-0.103	0.011	-0.056	-0.040	0.002	-0.037
Nautiliales	0.070	0.005	0.048	0.211	0.045	0.107
NB1-j	0.226	0.051	0.244	0.165	0.027	0.119
Neisseriales	0.083	0.007	0.118	-0.055	0.003	-0.136
Nitrosomonadales	0.170	0.029	0.129	0.160	0.026	0.238
Nitrososphaerales	-0.219	0.048	-0.152	-0.070	0.005	-0.103
Nitrospirales	-0.421	0.178	-0.257	0.068	0.005	0.094
Nostocales	0.055	0.003	0.013	0.085	0.007	0.044
Oceanospirillales	0.099	0.010	0.142	-0.011	0.000	-0.121
OM190	0.179	0.032	0.112	0.051	0.003	0.000
Opitutales	0.135	0.018	0.128	0.093	0.009	0.022
Oscillatoriales	0.074	0.006	-0.032	0.032	0.001	0.043
Pasteurellales	0.192	0.037	0.186	-0.047	0.002	-0.071
Phycisphaerales	0.161	0.026	0.206	-0.080	0.006	-0.127
Pirellulales	0.187	0.035	0.162	0.154	0.024	0.049
Planctomycetales	0.039	0.002	0.199	0.251	0.063	0.223
Pseudomonadales	-0.042	0.002	0.058	0.210	0.044	0.001
Rhizobiales	0.162	0.026	0.111	0.015	0.000	0.077
Rhodobacterales	0.018	0.000	0.014	-0.034	0.001	-0.090
Rhodocyclales	-0.380	0.144	-0.174	0.041	0.002	-0.050
Rhodospirillales	-0.016	0.000	0.022	0.141	0.020	0.009
Rickettsiales	0.102	0.010	0.101	0.049	0.002	0.095
Roseiflexales	0.248	0.061	0.142	-0.082	0.007	0.026
Rubrobacterales	0.000	0.000	0.064	-0.109	0.012	-0.101
S0208	0.111	0.012	-0.014	-0.039	0.002	-0.068
S085	-0.155	0.024	-0.102	-0.133	0.018	-0.126

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Sediment-1	0.054	0.003	0.160	-0.275	0.076	-0.091
SJA-36	0.110	0.012	0.043	0.085	0.007	0.104
SM1D11	0.000	0.000	0.000	0.000	0.000	0.000
Solibacterales	0.000	0.000	0.016	0.298	0.089	0.159
Solirubrobacterales	0.148	0.022	0.092	0.100	0.010	0.044
Spartobacteriales	-0.040	0.002	-0.042	0.179	0.032	0.202
Sphingobacteriales	0.144	0.021	0.180	0.051	0.003	0.004
Sphingomonadales	-0.140	0.020	-0.165	-0.084	0.007	-0.045
Stramenopiles	-0.179	0.032	-0.053	-0.100	0.010	-0.036
Streptophyta	-0.007	0.000	-0.010	0.006	0.000	-0.040
Sva0725	0.009	0.000	-0.067	-0.018	0.000	-0.040
Synergistales	0.030	0.001	0.020	0.213	0.045	0.130
Syntrophobacterales	-0.308	0.095	-0.232	0.018	0.000	-0.067
Thermoanaerobacterales	-0.046	0.002	-0.081	0.190	0.036	0.166
Thermobaculales	-0.175	0.030	-0.127	-0.047	0.002	-0.128
Thermomicrobiales	-0.146	0.021	0.062	0.045	0.002	-0.091
Thermoplasmatales	0.000	0.000	0.000	0.000	0.000	0.000
Thermotogales	-0.142	0.020	0.013	-0.060	0.004	-0.056
Thiotrichales	0.018	0.000	0.012	-0.077	0.006	-0.085
TIBE07	0.097	0.009	0.069	-0.080	0.006	-0.053
Verrucomicrobiales	-0.176	0.031	-0.090	0.313	0.098	0.241
wb1_H11	0.002	0.000	0.009	0.039	0.001	0.021
WCHB1-50	0.000	0.000	0.000	0.000	0.000	0.000
Xanthomonadales	0.260	0.067	0.203	-0.004	0.000	0.081
Unclassified Bacteria	0.375	0.140	0.278	-0.026	0.001	0.035
Unclassified , Phylum *Planctomycetes*	-0.031	0.001	0.058	0.210	0.044	0.081
Unclassified, Phylum *Chloroflexi*	0.122	0.015	0.101	0.040	0.002	0.030

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Unclassified, Phylum *Cyanobacteria*	0.170	0.029	0.177	-0.047	0.002	-0.102
Unclassified, Phylum *Firmicutes*	0.000	0.000	0.000	0.000	0.000	0.000
Unclassified, Phylum *Proteobacteria*	0.168	0.028	0.091	-0.158	0.025	-0.116
Unnamed, Phylum *Acidobacteria*	-0.199	0.040	-0.164	-0.289	0.084	-0.198
Unnamed, Phylum *Bacteroidetes*	-0.156	0.024	-0.259	0.295	0.087	0.365
Unnamed, Phylum CCM11b	-0.503	0.253	-0.337	-0.237	0.056	-0.069
Unnamed, Phylum *Chloroflexi*	0.029	0.001	0.018	-0.023	0.001	-0.004
Unnamed, Phylum GAL15	-0.061	0.004	0.032	-0.125	0.016	-0.058
Unnamed, Phylum GN02	-0.016	0.000	-0.026	0.087	0.007	0.064
Unnamed, Phylum GN12	0.167	0.028	0.220	-0.014	0.000	0.021
Unnamed, Phylum *Lentisphaerae*	-0.066	0.004	0.040	-0.046	0.002	-0.022
Unnamed, Phylum NKB19	0.114	0.013	0.121	0.050	0.003	0.135
Unnamed, Phylum OP3	0.117	0.014	0.114	-0.018	0.000	-0.003
Unnamed, Phylum *Proteobacteria*	0.000	0.000	0.000	0.000	0.000	0.000
Unnamed, Phylum SC3	0.151	0.023	0.211	-0.110	0.012	-0.102
Unnamed, Phylum SC4	-0.081	0.007	-0.107	0.062	0.004	0.084
Unnamed, Phylum SM2F11	-0.040	0.002	-0.100	0.117	0.014	0.100
Unnamed, Phylum ZB2	-0.377	0.142	-0.257	-0.056	0.003	-0.074
Unnamed, Phylum SR1	0.120	0.015	0.134	-0.031	0.001	-0.068
Unnamed, Phylum *Verrucomicrobia*	-0.146	0.021	-0.135	0.041	0.002	0.059
Unnamed,Phylum WPS-2	-0.001	0.000	-0.053	0.118	0.014	0.146
Unclassified, Class *Acidobacteria*	-0.222	0.049	-0.116	-0.168	0.028	-0.180
Unclassified, Class *Anaerolineae*	0.045	0.002	0.021	-0.068	0.005	-0.062
Unclassified, Class *Betaproteobacteria*	0.090	0.008	0.056	0.080	0.006	0.040
Unclassified, Class *Deltaproteobacteria*	-0.103	0.011	-0.069	0.079	0.006	0.086
Unclassified, Class *Gammaproteobacteria*	-0.115	0.013	-0.117	0.136	0.019	0.124
Unclassified, Class RA13C7	-0.035	0.001	-0.002	-0.004	0.000	-0.059

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Unclassified, Class *Thermomicrobia*	-0.220	0.048	-0.167	-0.168	0.028	-0.195
Unclassified, Class TK17	-0.007	0.000	-0.006	0.262	0.068	0.147
Unnamed, Class 0319-6G9	0.222	0.050	0.174	-0.075	0.006	-0.079
Unnamed, Class 5B-18	-0.092	0.008	-0.010	-0.149	0.022	-0.106
Unnamed, Class ABS-6	0.072	0.005	-0.003	0.065	0.004	0.088
Unnamed, Class *Acidobacteria-5*	0.092	0.008	0.044	0.081	0.007	0.025
Unnamed, Class *Actinobacteria*	-0.282	0.080	-0.095	-0.134	0.018	0.026
Unnamed, Class *Alphaproteobacteria*	0.171	0.029	0.136	0.091	0.008	0.098
Unnamed, Class *Alphaproteobacteria*	-0.042	0.002	0.016	0.232	0.054	0.219
Unnamed, Class *Anaerolineae*	-0.153	0.024	0.030	0.140	0.020	-0.136
Unnamed, Class *Betaproteobacteria*	0.334	0.111	0.275	0.081	0.006	0.155
Unnamed, Class C6	0.057	0.003	0.036	-0.091	0.008	-0.084
Unnamed, Class CH21	0.134	0.018	0.122	-0.045	0.002	-0.067
Unnamed, Class *Chloracidobacteria*	0.016	0.000	-0.002	0.100	0.010	0.116
Unnamed, Class *Chloroflexi*	-0.134	0.018	-0.150	-0.125	0.016	-0.104
Unnamed, Class *Dehalococcoidetes*	-0.180	0.032	-0.135	-0.123	0.015	-0.102
Unnamed, Class *Deltaproteobacteria*	-0.192	0.037	-0.068	0.174	0.030	0.049
Unnamed, Class *Epsilonproteobacteria*	-0.118	0.014	0.039	-0.323	0.104	-0.174
Unnamed, Class FFCH393	-0.224	0.050	-0.001	-0.255	0.065	-0.133
Unnamed, Class FFCH6980	0.222	0.049	0.115	0.107	0.011	0.105
Unnamed, Class *Flavobacteria*	-0.154	0.024	-0.027	-0.063	0.004	-0.003
Unnamed, Class *Gemmatimonadetes*	0.171	0.029	0.110	-0.065	0.004	-0.078
Unnamed, Class GKS2-174	0.068	0.005	0.056	-0.022	0.000	-0.040
Unnamed, Class GN07	-0.156	0.024	-0.127	-0.324	0.105	-0.179
Unnamed, Class GN08	0.137	0.019	0.179	-0.026	0.001	-0.042
Unnamed, Class koll11	0.113	0.013	0.068	-0.170	0.029	-0.145
Unnamed, Class *Kueneniae*	-0.009	0.000	0.028	-0.182	0.033	-0.139

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Unnamed, Class MJK10	0.054	0.003	0.086	0.050	0.003	0.025
Unnamed, Class MVS-40	0.265	0.070	0.223	-0.090	0.008	-0.150
Unnamed, Class OPB56	0.119	0.014	0.121	-0.038	0.001	-0.088
Unnamed, Class OPB80	0.156	0.024	0.154	-0.047	0.002	-0.057
Unnamed, Class *Opitutae*	0.175	0.031	0.128	-0.043	0.002	-0.008
Unnamed, Class PAUC37f	0.087	0.008	-0.001	0.033	0.001	0.017
Unnamed, Class *Phycisphaerae*	-0.060	0.004	0.044	0.140	0.019	0.035
Unnamed, Class PRR-11	-0.126	0.016	-0.075	-0.021	0.000	-0.079
Unnamed, Class PRR-12	0.080	0.006	0.150	-0.178	0.032	-0.171
Unnamed, Class PW285	0.141	0.020	0.148	0.047	0.002	0.082
Unnamed, Class PW285	-0.008	0.000	-0.049	0.040	0.002	0.081
Unnamed, Class RB25	0.053	0.003	0.128	-0.072	0.005	-0.089
Unnamed, Class RB384	0.111	0.012	0.101	0.008	0.000	0.062
Unnamed, Class S15B-MN24	0.089	0.008	0.017	0.089	0.008	0.067
Unnamed, Class S1a-1H	-0.192	0.037	-0.196	0.184	0.034	0.109
Unnamed, Class SBRH58	0.000	0.000	0.000	0.000	0.000	0.000
Unnamed, Class SC72	0.058	0.003	0.028	0.094	0.009	0.126
Unnamed, Class SJA-176	0.000	0.000	0.000	0.000	0.000	0.000
Unnamed, Class SJA-176	0.106	0.011	-0.002	0.052	0.003	-0.030
Unnamed, Class SJA-28	-0.192	0.037	-0.084	0.003	0.000	0.002
Unnamed, Class SM1B09	0.087	0.008	0.083	0.012	0.000	-0.079
Unnamed, Class SOGA31	-0.205	0.042	-0.117	-0.089	0.008	-0.074
Unnamed, Class *Thermomicrobia*	-0.171	0.029	-0.091	-0.123	0.015	-0.057
Unnamed, Class TK17	0.113	0.013	0.049	0.043	0.002	0.043
Unnamed, Class vadinHA49	0.021	0.000	-0.076	-0.113	0.013	-0.096
Unnamed, Class Verruco-5	0.228	0.052	0.204	-0.058	0.003	-0.045

Table 5.10 Correlations between bacterial orders and NMS axes for the B horizon ordination.

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
0319-7L14	-0.156	0.024	-0.111	-0.254	0.064	-0.241
32-20	0.074	0.005	0.030	0.048	0.002	-0.043
A31	0.088	0.008	-0.033	0.043	0.002	0.052
A4b	-0.095	0.009	-0.046	0.176	0.031	0.074
Acholeplasmatales	-0.249	0.062	-0.175	-0.104	0.011	-0.127
Acidimicrobiales	0.002	0.000	-0.009	-0.107	0.012	-0.101
Acidobacteriales	-0.067	0.004	-0.014	0.099	0.010	-0.036
Actinomycetales	-0.019	0.000	-0.017	0.071	0.005	-0.036
AKYG885	-0.177	0.031	-0.069	-0.172	0.029	-0.259
Anaerolineales	-0.216	0.046	-0.056	-0.072	0.005	-0.049
Aquificales	0.177	0.031	0.191	-0.014	0.000	-0.088
Armatimonadales	-0.097	0.009	-0.129	0.081	0.006	0.069
B07_WMSP1	0.174	0.030	0.136	0.031	0.001	0.053
Bacillales	-0.074	0.005	-0.093	-0.198	0.039	-0.083
Bacteroidales	0.010	0.000	-0.056	0.025	0.001	-0.051
Bdellovibrionales	-0.011	0.000	-0.083	0.163	0.027	0.206
Brachyspirales	-0.009	0.000	-0.031	0.101	0.010	0.127
Burkholderiales	-0.062	0.004	-0.042	0.016	0.000	-0.149
Caldilineales	-0.245	0.060	-0.182	0.017	0.000	-0.048
Campylobacterales	-0.102	0.010	-0.084	-0.088	0.008	-0.065
Cardiobacteriales	-0.001	0.000	0.049	0.158	0.025	0.118
Caulobacterales	-0.221	0.049	0.013	-0.289	0.084	-0.013
CFB-26	0.123	0.015	0.056	0.176	0.031	0.138
Chlamydiales	-0.318	0.101	-0.230	-0.341	0.116	-0.266

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Chlorobiales	0.000	0.000	0.000	0.000	0.000	0.000
Chloroflexales	0.073	0.005	0.019	0.046	0.002	-0.068
Chlorophyta	-0.261	0.068	-0.251	0.126	0.016	-0.086
Chromatiales	-0.247	0.061	-0.032	-0.058	0.003	-0.027
Chroococcales	0.045	0.002	-0.003	0.076	0.006	0.100
Chthonomonadales	-0.113	0.013	0.001	-0.036	0.001	-0.014
CL500-15	0.009	0.000	0.007	0.004	0.000	-0.072
Clostridiales	0.287	0.082	0.216	-0.064	0.004	-0.006
Coriobacteriales	0.170	0.029	0.162	0.038	0.001	0.054
CTD005-82B-02	-0.149	0.022	-0.135	-0.116	0.013	-0.099
CV106	-0.187	0.035	-0.082	-0.085	0.007	-0.164
Deferribacterales	0.030	0.001	0.003	-0.003	0.000	-0.060
Dehalococcoidales	0.030	0.001	0.018	0.100	0.010	0.087
Deinococcales	0.085	0.007	0.059	0.057	0.003	0.085
Desulfuromonadales	0.175	0.031	0.160	0.023	0.001	0.061
Desulfovibrionales	-0.164	0.027	-0.247	0.118	0.014	0.059
Dictyoglomales	0.000	0.000	0.000	0.000	0.000	0.000
DS-18	-0.221	0.049	-0.170	-0.082	0.007	-0.045
Desulfurellales	0.000	0.000	0.000	0.000	0.000	0.000
Elusimicrobiales	-0.143	0.020	-0.079	0.357	0.127	0.293
Enterobacteriales	-0.086	0.007	-0.008	0.047	0.002	0.013
Entotheonellales	0.129	0.017	0.129	0.119	0.014	0.004
envOPS12	0.030	0.001	0.006	-0.208	0.043	-0.191
Euglenozoa	0.000	0.000	0.000	0.000	0.000	0.000
Euzebiales	-0.104	0.011	-0.138	-0.326	0.106	-0.186
Exiguobacterales	-0.028	0.001	0.031	0.033	0.001	-0.026
FAC88	-0.020	0.000	-0.040	0.001	0.000	-0.034

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Fibrobacterales	-0.033	0.001	0.021	0.145	0.021	0.039
Flavobacteriales	0.106	0.011	0.028	-0.072	0.005	0.004
GCA004	-0.018	0.000	-0.005	0.135	0.018	0.059
Gemata	-0.085	0.007	0.049	0.111	0.012	0.019
Gemmatimonadales	-0.232	0.054	-0.130	-0.132	0.017	-0.025
H39	0.068	0.005	0.061	0.089	0.008	0.055
Halanaerobiales	-0.140	0.020	-0.107	0.213	0.045	0.175
Halobacteriales	0.195	0.038	0.109	0.099	0.010	0.121
Herpetosiphonales	0.052	0.003	0.057	0.079	0.006	0.085
HN1-15	0.048	0.002	-0.075	0.131	0.017	0.075
Holophagales	0.163	0.027	0.118	0.053	0.003	0.028
Hydrogenophilales	0.133	0.018	0.130	-0.026	0.001	-0.093
koll13	-0.139	0.019	-0.071	0.138	0.019	-0.051
Lactobacillales	0.052	0.003	0.036	0.125	0.016	0.120
LD1-PA13	0.025	0.001	0.027	0.134	0.018	-0.034
Legionellales	0.192	0.037	0.103	0.084	0.007	0.165
Leptospirales	0.032	0.001	-0.007	0.016	0.000	0.022
MC47	0.109	0.012	0.045	0.274	0.075	0.152
Methylococcales	0.090	0.008	0.044	0.036	0.001	0.060
Methylophilales	-0.297	0.088	-0.222	0.016	0.000	-0.032
Methanosarcinales	0.000	0.000	0.000	0.000	0.000	0.000
Methylacidiphilales	0.000	0.000	-0.033	-0.008	0.000	-0.036
MIZ46	-0.126	0.016	-0.064	0.168	0.028	0.234
mle1-12	-0.003	0.000	-0.017	-0.050	0.003	0.012
mle1-48	-0.110	0.012	0.029	0.123	0.015	0.042
MVP-88	-0.180	0.033	-0.101	-0.026	0.001	-0.051
Mycoplasmatales	0.155	0.024	0.077	0.033	0.001	0.053

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Myxococcales	0.160	0.026	0.119	0.118	0.014	0.122
Natranaerobiales	0.000	0.000	0.000	0.000	0.000	0.000
Nautiliales	0.007	0.000	0.100	-0.114	0.013	-0.100
NB1-j	-0.106	0.011	-0.091	0.112	0.013	0.207
Neisseriales	-0.164	0.027	-0.090	0.088	0.008	-0.134
Nitrospirales	-0.159	0.025	-0.033	-0.280	0.079	-0.166
Nitrosomonadales	-0.318	0.101	-0.222	-0.075	0.006	-0.017
Nitrososphaerales	0.307	0.094	0.220	-0.082	0.007	-0.153
Nostocales	0.060	0.004	0.053	0.056	0.003	0.021
Oceanospirillales	-0.087	0.008	-0.084	0.083	0.007	0.014
OM190	0.021	0.000	0.078	0.260	0.068	0.210
Opitutales	0.072	0.005	0.193	0.277	0.077	0.221
Oscillatoriales	-0.010	0.000	-0.045	0.142	0.020	0.147
Pasteurellales	-0.229	0.053	-0.148	0.092	0.008	0.113
Phycisphaerales	-0.041	0.002	-0.064	0.126	0.016	0.021
Pirellulales	-0.170	0.029	-0.073	0.085	0.007	-0.102
Planctomycetales	0.079	0.006	0.097	0.165	0.027	0.189
Pseudomonadales	0.022	0.000	0.006	-0.164	0.027	-0.081
Rhodocyclales	-0.283	0.080	-0.245	-0.072	0.005	0.087
Rhizobiales	0.316	0.100	0.194	0.246	0.060	0.199
Rhodobacterales	-0.049	0.002	0.026	0.153	0.023	0.005
Rhodospirillales	0.020	0.000	0.062	0.088	0.008	0.081
Rickettsiales	0.206	0.042	0.192	-0.005	0.000	-0.075
Roseiflexales	0.087	0.008	0.003	0.061	0.004	0.033
Rubrobacterales	0.046	0.002	-0.077	-0.021	0.000	-0.100
S0208	-0.240	0.057	-0.162	0.005	0.000	-0.075
S085	-0.215	0.046	-0.132	0.089	0.008	0.012

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Sediment-1	0.110	0.012	0.106	0.116	0.013	0.068
SJA-36	0.177	0.032	0.179	-0.103	0.011	-0.053
SM1D11	-0.161	0.026	-0.120	0.150	0.022	0.148
Solibacterales	-0.164	0.027	-0.077	0.158	0.025	0.044
Solirubrobacterales	-0.178	0.032	-0.014	-0.068	0.005	-0.143
Spartobacteriales	0.161	0.026	0.193	0.069	0.005	0.086
Sphingobacteriales	-0.043	0.002	-0.034	0.179	0.032	0.231
Sphingomonadales	-0.135	0.018	-0.043	-0.010	0.000	-0.017
Stramenopiles	-0.086	0.007	-0.083	0.087	0.008	0.075
Streptophyta	0.132	0.017	0.168	0.031	0.001	0.058
Sva0725	0.007	0.000	0.137	-0.145	0.021	-0.026
Synergistales	-0.246	0.060	-0.236	0.050	0.002	0.022
Syntrophobacterales	-0.069	0.005	-0.090	-0.028	0.001	-0.107
Thermobaculales	0.044	0.002	0.073	0.080	0.006	0.073
Thermotogales	0.100	0.010	0.069	0.164	0.027	0.026
Thermomicrobiales	-0.292	0.085	-0.128	-0.077	0.006	-0.021
Thermoplasmatales	-0.140	0.020	-0.107	0.213	0.045	0.175
Thermoanaerobacterales	0.000	0.000	0.000	0.000	0.000	0.000
Thiotrichales	-0.209	0.044	-0.057	-0.116	0.013	0.034
TIBE07	0.023	0.001	0.003	-0.041	0.002	0.052
Verrucomicrobiales	-0.073	0.005	0.052	0.147	0.022	0.192
wb1_H11	-0.149	0.022	-0.158	-0.063	0.004	-0.054
WCHB1-50	-0.056	0.003	-0.032	-0.093	0.009	-0.097
Xanthomonadales	0.084	0.007	0.041	0.054	0.003	0.176
Unnamed, Phylum *Acidobacteria*	-0.063	0.004	-0.016	-0.061	0.004	-0.082
Unnamed, Phylum *Bacteroidetes*	0.089	0.008	0.015	-0.041	0.002	-0.051
Unnamed, Phylum CCM11b	-0.199	0.040	-0.124	-0.347	0.121	-0.317

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Unnamed, Phylum *Chloroflexi*	0.041	0.002	0.029	0.127	0.016	0.124
Unclassified, Phylum *Chloroflexi*	-0.101	0.010	-0.107	0.099	0.010	0.040
Unclassified, Phylum *Cyanobacteria*	0.000	0.000	0.000	0.000	0.000	0.000
Unclassified, Phylum *Firmicutes*	0.145	0.021	0.093	-0.016	0.000	-0.083
Unnamed, Phylum GN02	0.142	0.020	0.101	0.133	0.018	0.113
Unnamed, Phylum GN12	-0.062	0.004	-0.061	0.151	0.023	0.104
Unnamed, Phylum GAL15	-0.209	0.044	-0.113	-0.305	0.093	-0.241
Unnamed, Phylum *Lentisphaerae*	0.000	0.000	0.000	0.000	0.000	0.000
Unnamed, Phylum NKB19	0.222	0.049	0.236	0.053	0.003	0.091
Unnamed, Phylum OP3	-0.085	0.007	-0.104	-0.072	0.005	0.028
Unclassified , Phylum *Planctomycetes*	-0.092	0.008	-0.064	0.027	0.001	0.025
Unclassified, Phylum *Proteobacteria*	-0.341	0.116	-0.261	-0.255	0.065	-0.167
Unnamed, Phylum *Proteobacteria*	0.203	0.041	0.184	0.029	0.001	0.030
Unnamed, Phylum SC3	-0.027	0.001	0.012	0.163	0.027	0.167
Unnamed, Phylum SC4	0.082	0.007	0.088	0.082	0.007	0.118
Unnamed, Phylum SM2F11	-0.017	0.000	-0.053	0.008	0.000	-0.043
Unnamed, Phylum SR1	0.000	0.000	0.000	0.000	0.000	0.000
Unnamed, Phylum *Verrucomicrobia*	0.244	0.060	0.144	0.043	0.002	0.059
Unnamed,Phylum WPS-2	-0.238	0.057	-0.161	-0.130	0.017	-0.134
Unnamed, Phylum ZB2	0.045	0.002	-0.003	0.076	0.006	0.100
Unclassified Bacteria	0.075	0.006	0.096	0.163	0.026	0.124
Unclassified, Class *Acidobacteria*	-0.008	0.000	-0.050	0.002	0.000	-0.011
Unclassified, Class *Anaerolineae*	0.036	0.001	0.036	0.047	0.002	0.034
Unclassified, Class *Betaproteobacteria*	-0.009	0.000	-0.038	0.037	0.001	0.072
Unclassified, Class *Deltaproteobacteria*	0.025	0.001	-0.025	0.126	0.016	0.162
Unclassified, Class *Gammaproteobacteria*	0.000	0.000	0.000	0.000	0.000	0.000
Unclassified, Class RA13C7	0.000	0.000	0.000	0.000	0.000	0.000

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Unclassified, Class *Thermomicrobia*	0.000	0.000	0.000	0.000	0.000	0.000
Unclassified, Class TK17	0.000	0.000	0.000	0.000	0.000	0.000
Unnamed, Class 0319-6G9	-0.142	0.020	-0.091	-0.173	0.030	-0.179
Unnamed, Class 5B-18	-0.156	0.024	-0.153	-0.046	0.002	-0.120
Unnamed, Class ABS-6	0.127	0.016	0.057	0.044	0.002	0.146
Unnamed, Class *Acidobacteria-5*	0.182	0.033	0.202	-0.210	0.044	-0.145
Unnamed, Class *Actinobacteria*	0.171	0.029	0.124	-0.154	0.024	-0.096
Unnamed, Class *Alphaproteobacteria*	0.007	0.000	0.030	0.188	0.035	0.163
Unnamed, Class *Alphaproteobacteria*	0.003	0.000	0.069	0.042	0.002	0.106
Unnamed, Class *Anaerolineae*	-0.170	0.029	0.058	-0.177	0.031	-0.052
Unnamed, Class *Betaproteobacteria*	0.159	0.025	0.164	0.269	0.072	0.232
Unnamed, Class C6	0.115	0.013	0.022	0.084	0.007	0.114
Unnamed, Class CH21	-0.025	0.001	0.026	0.061	0.004	0.026
Unnamed, Class *Chloracidobacteria*	0.081	0.007	0.018	0.091	0.008	0.113
Unnamed, Class *Chloroflexi*	-0.059	0.003	-0.024	-0.046	0.002	-0.131
Unnamed, Class *Dehalococcoidetes*	-0.229	0.053	-0.148	0.092	0.008	0.113
Unnamed, Class *Deltaproteobacteria*	-0.005	0.000	-0.071	-0.095	0.009	-0.085
Unnamed, Class *Epsilonproteobacteria*	-0.039	0.002	-0.022	-0.355	0.126	-0.115
Unnamed, Class FFCH393	-0.063	0.004	0.019	0.049	0.002	0.015
Unnamed, Class FFCH6980	0.070	0.005	0.128	0.184	0.034	0.135
Unnamed, Class *Flavobacteria*	-0.052	0.003	0.032	-0.008	0.000	0.123
Unnamed, Class *Gemmatimonadetes*	-0.156	0.024	-0.051	-0.102	0.010	-0.183
Unnamed, Class GKS2-174	-0.038	0.001	0.053	-0.121	0.015	0.025
Unnamed, Class GN07	-0.133	0.018	-0.030	-0.011	0.000	-0.018
Unnamed, Class GN08	-0.045	0.002	-0.059	0.214	0.046	0.118
Unnamed, Class koll11	-0.121	0.015	-0.108	-0.101	0.010	-0.046
Unnamed, Class *Kueneniae*	-0.277	0.077	-0.086	-0.352	0.124	-0.233

	Axis 1			Axis 2		
Order	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Unnamed, Class MJK10	0.104	0.011	0.034	0.134	0.018	0.128
Unnamed, Class MVS-40	-0.116	0.013	-0.113	-0.008	0.000	-0.038
Unnamed, Class OPB56	0.000	0.000	0.000	0.000	0.000	0.000
Unnamed, Class OPB80	-0.237	0.056	-0.192	-0.191	0.036	-0.187
Unnamed, Class *Opitutae*	-0.072	0.005	0.002	-0.108	0.012	-0.187
Unnamed, Class PAUC37f	-0.017	0.000	-0.011	-0.103	0.011	-0.106
Unnamed, Class *Phycisphaerae*	-0.253	0.064	-0.136	-0.134	0.018	-0.111
Unnamed, Class PRR-11	0.079	0.006	0.015	0.119	0.014	0.083
Unnamed, Class PRR-12	0.130	0.017	0.043	-0.044	0.002	-0.119
Unnamed, Class PW285	-0.186	0.035	-0.124	0.211	0.045	0.181
Unnamed, Class PW285	0.062	0.004	-0.017	-0.150	0.023	-0.110
Unnamed, Class RB25	0.101	0.010	0.035	0.158	0.025	0.135
Unnamed, Class RB384	0.196	0.039	0.245	0.021	0.000	0.020
Unnamed, Class S15B-MN24	0.005	0.000	-0.059	0.124	0.015	0.091
Unnamed, Class S1a-1H	-0.179	0.032	-0.139	0.133	0.018	0.050
Unnamed, Class SBRH58	-0.229	0.053	-0.148	0.092	0.008	0.113
Unnamed, Class SC72	0.112	0.013	0.091	-0.041	0.002	-0.034
Unnamed, Class SJA-176	0.077	0.006	0.037	-0.019	0.000	-0.095
Unnamed, Class SJA-176	0.037	0.001	0.066	-0.071	0.005	0.003
Unnamed, Class SJA-28	0.052	0.003	0.039	-0.074	0.005	-0.059
Unnamed, Class SM1B09	-0.149	0.022	-0.172	0.242	0.058	0.166
Unnamed, Class SOGA31	-0.175	0.031	-0.095	-0.166	0.027	-0.168
Unnamed, Class *Thermomicrobia*	-0.102	0.010	-0.223	-0.267	0.071	-0.293
Unnamed, Class TK17	-0.164	0.027	-0.068	0.192	0.037	0.124
Unnamed, Class vadinHA49	-0.192	0.037	0.039	0.123	0.015	0.015
Unnamed, Class Verruco-5	0.077	0.006	0.078	0.125	0.016	0.142